The impact of dietary interventions for irritable bowel syndrome on the gastrointestinal microbiota, symptoms, nutrient intake and quality of life

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The impact of dietary interventions for irritable bowel syndrome on the gastrointestinal microbiota, symptoms, nutrient intake and quality of life

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For Gramps, Nanna,
Papi, Mum, Natalie, Aimi, Alexander

Abstract

Irritable bowel syndrome (IBS) is a common functional bowel disorder characterised by abdominal pain or discomfort and altered bowel habit. It negatively influences quality of life and poses a considerable economic burden to patients, healthcare resources and society. Treatments are symptom-directed but the complex pathophysiology of the condition, and the heterogeneity and instability of presenting symptoms lead to treatment challenges. Many patients with IBS have attempted and/or seek information about dietary approaches to manage their symptoms.

One issue that has limited obtaining robust research evidence for dietary advice interventions in IBS is the difficulty of implementing a suitable placebo control. Previous studies have utilised standard IBS dietary advice or habitual diet as the placebo comparator intervention, or have been feeding studies, all of which have their limitations. Therefore, a novel sham diet was designed, developed and evaluated for use as a placebo control in a dietary advice RCT.

There is growing evidence for the clinical effectiveness of the low FODMAP diet in IBS, however a blinded, placebo-controlled, dietary advice RCT had never been performed. Furthermore, whether the acute effect of the low FODMAP diet on the GI microbiota could be prevented required investigation. Therefore a 2x2 factorial design blinded placebo-controlled RCT was conducted in 104 patients. More patients in the low FODMAP diet group reported a clinically important reduction in IBS-SSS score compared with the sham diet group (73% vs 42%, p=0.005). There was also a lower abundance of stool Bifidobacteria in the low FODMAP diet group compared with sham (8.8 vs 9.0 $\log_{10}$ cells/g faeces, p=0.028). Probiotic co-administration with VSL#3 ameliorated the effect of the low FODMAP diet on Bifidobacteria, and as a sole intervention increased Bifidobacteria abundance compared with placebo but did not have a convincing effect on GI symptoms.

In conclusion, there is compelling evidence for the beneficial effect of the low FODMAP diet in a majority of patients with IBS. The ramifications of this diet both on the GI microbiota and on nutrient intake confirm the importance of FODMAP reintroduction to tolerance, and that the diet is dietitian-led. Probiotic ameliorated the effect of low FODMAP diet-induced microbiota aberration, however the degree of recovery in response to FODMAP reintroduction requires evaluation before probiotic co-administration is recommended as routine practice.

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Papers and reviews:

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Staudacher H.M. & Gibson P.R. 2015. How healthy is a gluten-free diet? *Br J Nutr*, 114 (10), 1539-1541.

Staudacher H.M. 2015. Probiotics for lactose intolerance and irritable bowel syndrome. *Brit J Comm Nurs,* 20 (Supp6a), S12-4.

Whigham L., Joyce T., Harper G., Irving P.M., **Staudacher H.M.**, Whelan K. & Lomer M.C.E. 2015. Clinical effectiveness and economic costs of group versus one-to-one education for short-chain fermentable carbohydrate restriction (low FODMAP diet) in the management of irritable bowel syndrome. *J Hum Nutr Diet,* 28(6), 687-696.

Staudacher H.M., Irving P.M., Lomer M.C.E. & Whelan K. 2014. Mechanisms and efficacy of dietary FODMAP restriction in IBS. *Nat Rev Gastroenterol Hepatol,* 11, 256-66.

Staudacher H.M., Lomer M.C.E., Anderson J.L., Barrett J.S., Muir J.G., Irving P.M. & Whelan K. 2012.Fermentable carbohydrate restriction reduces luminal Bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *J Nutr,* 142, 1510-1518.

Book chapter:

Staudacher H.M. & Parkes G. 2014. Irritable bowel syndrome: dietary management. In: Lomer, M.C.E. (Ed) *Advanced Nutrition and Dietetics in Gastroenterology.* John Wiley and Sons: 233-242.

Conference abstracts:

Staudacher H.M., Lomer M.C.E., Lindsay J.O., Irving P.M. & Whelan K. 2015. The impact of low FODMAP dietary advice and probiotics on symptoms in irritable bowel syndrome: a randomised, placebo-controlled, 2 × 2 factorial trial. *Gut,* 64(Suppl 1), A51.1-51

Martin L., van Vuuren C., Seamark L., Williams M., **Staudacher H.M.**, Irving P.M., Whelan K. & Lomer M.C.E. 2015 Long term effectiveness of short chain fermentable carbohydrate (FODMAP) restriction in patients with irritable bowel syndrome. *Gut,* 64(Suppl 1), A51.2-52

Staudacher H.M., Ross F.S., Briscoe Z.M. Irving, P.M., Whelan, K. & Lomer, M.C. E. 2015. Advice from a dietitian regarding the low FODMAP diet broadly maintains nutrient intake and does not alter fibre intake *Gut*, 64 (Suppl 1), A143-44.

Staudacher H.M., Lomer M.C.E., Anderson J.G., Barrett J.G., Muir J.S., Irving P.M. & Whelan K. 2014. Prebiotic intake in habitual diet is not associated with luminal Bifidobacteria concentration in irritable bowel syndrome *Proc Nutr Soc,* 73, E20.

Joyce T., **Staudacher H.M.**, Whelan K., Irving P.M. & Lomer M.C.E. 2014. Symptom response following advice on a diet low in short-chain fermentable carbohydrates (FODMAPs) for functional bowel symptoms in patients with IBD. *Gut*, 63(Suppl1), A164.

Joyce T., **Staudacher H.M.**, Whelan K., Irving P.M. & Lomer M.C.E. 2013. Group education is as effective as one-to-one sessions when administering the low FODMAP diet in functional bowel disorders. *Gut*, 62(Suppl 1), A276.

Staudacher H.M., Lomer M.C.E., Anderson J.L., Barrett J.S., Muir J.G., Irving P.M. & Whelan K. 2012. Impact of a fermentable carbohydrate restricted diet on luminal microbiota, fermentation, symptoms and nutrient intake in patients with irritable bowel syndrome: a randomised controlled trial. *Gut*, 61(2), A24.

Staudacher H.M., Lomer M.C.E., Anderson J.L., Irving P.M. & Whelan K. 2012. Impact of a diet low in fermentable carbohydrates on gastrointestinal symptoms, stool output and faecal pH in patients with irritable bowel syndrome: a randomised controlled trial. *Proc Nutr Soc*, 70, OCE5 (E293).

Awards:

2015 Nutrition Society Summer Meeting Postgraduate Competition Overall Winner. 'Dietary manipulation of the gastrointestinal microbiota in irritable bowel syndrome'

2015 European Nutrition Leadership Platform writing competition 'The paleo diet: A cave-man diet in the modern era'

2013 Rose Simmonds Award, British Dietetic Association. Best publication.
Staudacher **H.M.**, Lomer M.C., Anderson J.L., Barrett J.S., Muir J.G., Irving P.M. & Whelan K. 2012. Fermentable carbohydrate restriction reduces luminal Bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *J Nutr,* 142, 1510-1518.

List of abbreviations

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BSFS	Bristol Stool Form Scale
CI	Confidence interval
C_q	Threshold cycle
DP	Degree of polymerisation
DNA	Deoxyribonucleic acid
DRV	Dietary reference value
FISH	Fluorescence *in situ* hybridisation
FODMAP	Fermentable oligosaccharides, disaccharides, monosaccharides and polyols
FFQ	Food frequency questionnaire
FBD	Functional bowel disorder
FOS	Fructo-oligosaccharides
GI	Gastrointestinal
GLC	Gas liquid chromatography
GOS	Galacto-oligosaccharides
GSRS	Gastrointestinal system rating scale
HRQOL	Health-related quality of life
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IBS-C	Constipation-predominant irritable bowel syndrome
IBS-D	Diarrhoea-predominant irritable bowel syndrome
IBS-M	Mixed subtype irritable bowel syndrome
IBS-QOL	Irritable bowel syndrome quality of life questionnaire
IBS-SSS	Irritable bowel syndrome severity scoring system
IBS-U	Unsubtyped irritable bowel syndrome
LMT	Lysing matrix tube
MBH_20	Molecular biology grade water
MCID	Minimal clinically important difference
MRI	Magnetic resonance imaging
NDNS	National diet and nutrition survey
NNT	Number needed to treat
NSP	Nonstarch polysaccharides
NTC	No-template control
OR	Odds ratio
PI-IBS	Post-infectious irritable bowel syndrome
PP	Per protocol
qPCR	Quantitative polymerase chain reaction
RCT	Randomised controlled trial
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SCFA	Short chain fatty acid
SD	Standard deviation
SF-36	Short form 36 healthy survey questionnaire
VAS	Visual analogue scale

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1 Introduction

1.1 Irritable bowel syndrome

1.1.1 Introduction

Functional bowel disorders (FBD) are characterised by chronic lower gastrointestinal (GI) symptoms in the absence of alarm features that suggest the presence of other disease (Longstreth et al., 2006, Gunnarsson and Simren, 2008). Symptom duration of at least 6 months is necessary for diagnosis in order to distinguish FBD from transient GI symptoms. The Rome III diagnostic criteria for FBD are governed by the Rome Foundation, a not-for-profit expert panel devoted to the diagnosis and treatment of FBD. The following section will describe the diagnostic criteria, impact, pathogenesis and treatment of irritable bowel syndrome (IBS), one of the most prevalent FBD.

1.1.2 Diagnosis and prevalence

The diagnosis of IBS requires the presence of abdominal pain or discomfort together with an alteration in stool output (**Figure 1.1**) (Longstreth et al., 2006). Prior to a diagnosis of IBS, inflammatory markers and coeliac serology should be performed to rule out other causes for symptoms, and 'red flag' symptoms, such as rectal bleeding, should be investigated further (NICE, 2015).

A clinical diagnosis of IBS has traditionally been one characterised by exclusion of other conditions. Alternatively, guidelines encourage that a positive diagnosis is made based on clinical history and the Rome III criteria. However, diagnosis of IBS is inherently difficult given the heterogeneity of the condition and symptom overlap with organic pathology, such as coeliac disease. Hence there has been recent research interest in the identification of diagnostic biomarkers for IBS. None in isolation perform better than symptom-based diagnostic criteria, however combined biomarkers, psychological markers and symptom criteria may present a future opportunity for rapid diagnosis (Sood et al., 2015).

IBS is a common condition worldwide, contributing to 30% of general practitioner consultations related to GI complaints (Thompson et al., 2000) and up to 60% of referrals to gastroenterology outpatient clinics (Jones et al., 2000). It affects more females than males and is more prevalent in those under 40 years of age. A pooled global prevalence rate of 14% of females and 9% of males has been reported in a large systematic review and meta-analysis of 55 studies conducted across America, Asia, Europe and Africa (Lovell and Ford, 2012).

Rome III	NICE
Recurrent abdominal pain or discomfort*at least 3 days per month in the last 3 months associated with 2 or more of the following: (Criteria must be fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis)	Abdominal pain or discomfort that is relieved by defaecation or associated with altered bowel frequency or stool form. This should be accompanied by at least two of the following four symptoms:
1. Improvement with defaecation 2. Onset associated with a change in frequency of stool 3. Onset associated with a change in form (appearance) of stool	1. Altered stool passage (straining, urgency, incomplete evacuation) 2. Abdominal bloating, distension, tension or hardness 3. Symptoms made worse by eating 4. Passage of mucus
*Discomfort means an uncomfortable sensation not described as pain	Supporting symptoms: Lethargy, nausea, backache and bladder symptoms

Figure 1.1 Rome III (Longstreth et al., 2006) and NICE diagnostic criteria (NICE, 2015) for IBS

The Rome III criteria specify four different IBS subtypes based on predominant stool form. Subtypes may differ in their pathophysiology, highlighting the importance of subtyping patients for targeting treatment. Subtyping is performed using the Bristol Stool Form Scale (BSFS) (**Figure 1.2**) (O'Donnell et al., 1990). Patients with diarrhoea-predominant IBS (IBS-D) and constipation-predominant (IBS-C) subtypes, as their names suggest, are characterised by the extremes of stool form. Patients with mixed subtype (IBS-M) have both diarrhoea and constipation, and patients with unsubtyped IBS (IBS-U) generally pass normal stools (**Figure 1.3**) (Longstreth et al., 2006). IBS-D is generally reported as the most common subtype (40-60% of all IBS) (Yao et al., 2012, Engsbro et al., 2012). Despite the utility of distinct subtype classifications, stool output in IBS is unstable. Indeed, at least 50% of patients with IBS switch subtype over a short time frame, and this occurs predominantly in those with IBS-D or IBS-C (Mearin et al., 2004, Engsbro et al., 2012).

Although altered stool form and abdominal pain or discomfort are the hallmark features of IBS, other lower GI symptoms frequently co-exist, including bloating, flatulence, urgency and defaecation difficulties such as a sensation of incomplete evacuation. Indeed, UK clinical guidelines for IBS incorporate symptoms such as bloating and urgency as supportive of a diagnosis (**Figure 1.1**) (NICE, 2015). Other frequently reported comorbidities include upper GI symptoms, chronic pain syndromes (e.g. fibromyalgia), psychiatric conditions, somatisation and lethargy (Ladabaum et al., 2012, Whitehead et al., 2007). The high incidence of GI and

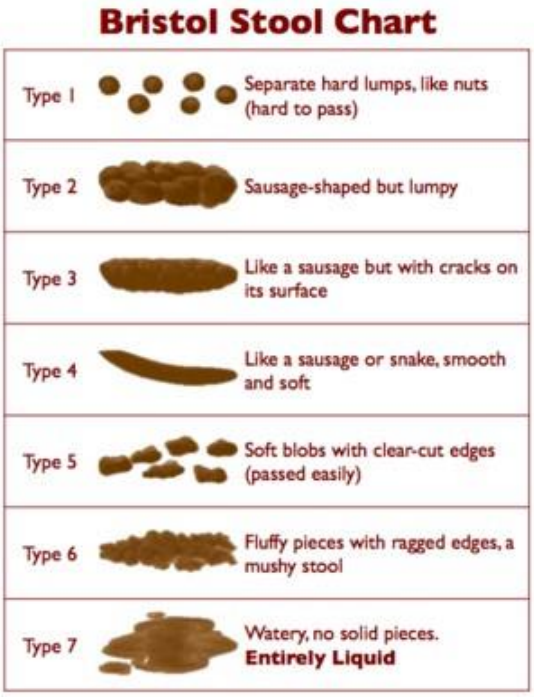

Figure 1.2 The Bristol Stool Form Scale categorises stool consistency into 7 types. Types 3,4,5 are considered normal stool consistency.

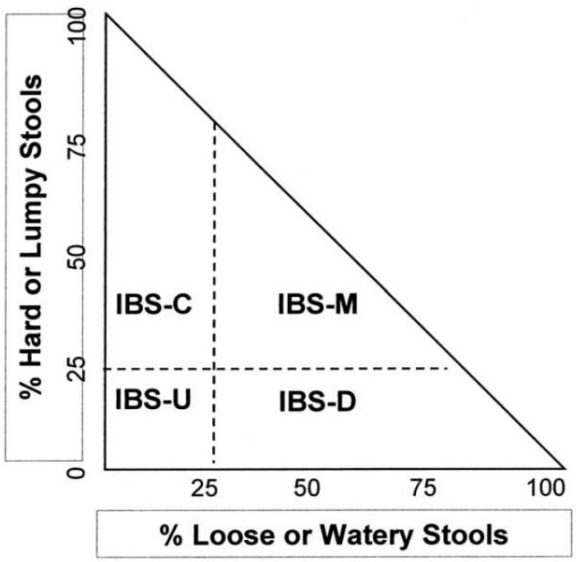

Figure 1.3 IBS subtypes based on stool form. IBS-D, diarrhoea-predominant IBS; IBS-C, constipation-predominant IBS; IBS-M, mixed subtype IBS; IBS-U unsubtyped IBS

extra-intestinal conditions in IBS compared with healthy individuals is proposed to be due to hypervigilance and a lower threshold for medical consultation (Whitehead et al., 2007).

1.1.3 Impact on health-related quality of life (HRQOL)

Health-related quality of life (HRQOL) has been defined as quality of life that relates to health, rather than factors such as income or freedom that might also be considered important (Guyatt et al., 1993). Although there is no impact of IBS on mortality, the morbidity associated with its chronic nature and the high incidence of co-existing GI and extra-intestinal conditions contributes to a negative impact on HRQOL (Chang et al., 2010). Specific concerns identified in a community survey of nearly 2,000 individuals with IBS from eight European countries were diet, concentration, sleep, coping with long journeys and their physical appearance (Hungin et al., 2003). Furthermore, patients with IBS spent more days in bed, missed more work days and spent more days seeing their doctor than individuals without the condition (Hungin et al., 2003).

Validated instruments for measuring HRQOL have been used to verify the negative impact of IBS on HRQOL. Patients with IBS report lower HRQOL scores for physical and mental domains compared with healthy controls according to a validated generic HRQOL questionnaire (SF-36) (Gralnek et al., 2000). Strikingly, lower subscores were also evident compared with patients with chronic disease such as diabetes, end stage renal disease and gastro-oesophageal reflux disease (Gralnek et al., 2000). Patients with IBS-D and IBS-M report worse HRQOL scores compared with IBS-C according to an IBS-specific HRQOL questionnaire (IBS-QOL), including lower subscores for food avoidance (Singh et al., 2015). Other determinants of lower HRQOL scores in IBS include symptom severity (more frequent diarrhoea, more severe abdominal pain), older age and psychological factors (depression, neuroticism) (Koloski et al., 2012), highlighting the importance of both physical and psychological symptoms in determining HRQOL in IBS.

1.1.4 Economic impact

The economic cost of a disease/disorder can be categorised into three domains. Firstly, there are the costs incurred by the patient for medications and loss of earnings associated with the condition. There is no data on annual patient-incurred costs in IBS. Secondly, there are direct healthcare costs incurred due to medical consultations, investigations, and emergency care, which totals between £90 and £316 annually per patient in the UK (Canavan et al., 2014). The

national annual direct national healthcare cost totals £45-200 million in the UK and more than half of the national direct costs are related to non-GI complaints (Canavan et al., 2014), underlining the considerable comorbidity evident in patients with IBS. Finally, there are societal costs relating to absenteeism. There is little recent data in IBS but up to 50% of patients require time off work for their condition in Europe (Canavan et al., 2014), which likely has considerable downstream effects on productivity and costs to the workplace. Overall, it is acknowledged that IBS places a significant cost burden directly on the patient, to healthcare resources and society, emphasising the importance of efficient diagnosis and development of low cost treatment measures.

1.1.5 Pathogenesis of IBS

The pathogenesis of IBS is incompletely understood but is known to be multifactorial and complex in nature (**Figure 1.4**). Although there appears to be some genetic potential for the development of IBS (Villani et al., 2010), most work on the aetiology of the condition has concentrated on the contribution of the central nervous system, such as altered brain-gut signalling and psychological distress, and altered peripheral regulation of gut function (Hungin et al., 2015). The following sections will describe the evidence for the contribution of each of these factors to the pathogenesis of IBS.

1.1.5.1 Central nervous system

Central nervous system alterations have been proposed to contribute to the pathophysiology of IBS, especially in those with severe symptoms (Drossman et al., 2011). Abnormalities in afferent processing and the activation of emotional arousal networks that modulate the afferent signals have been identified. There is also preliminary evidence for the presence of structural grey matter abnormalities in patients with IBS compared with healthy individuals, some of which are associated with anxiety and depression (Tillisch and Labus, 2011).

Along with these central alterations, accumulating evidence suggests that psychological stressors may have a direct role in the pathogenesis of IBS. For example, patients with IBS report a higher prevalence of early life trauma (e.g. physical, emotional or sexual abuse) than healthy controls, especially in females (Bradford et al., 2012). Furthermore, the two-fold higher prevalence of anxiety and depression in patients with IBS compared with healthy controls

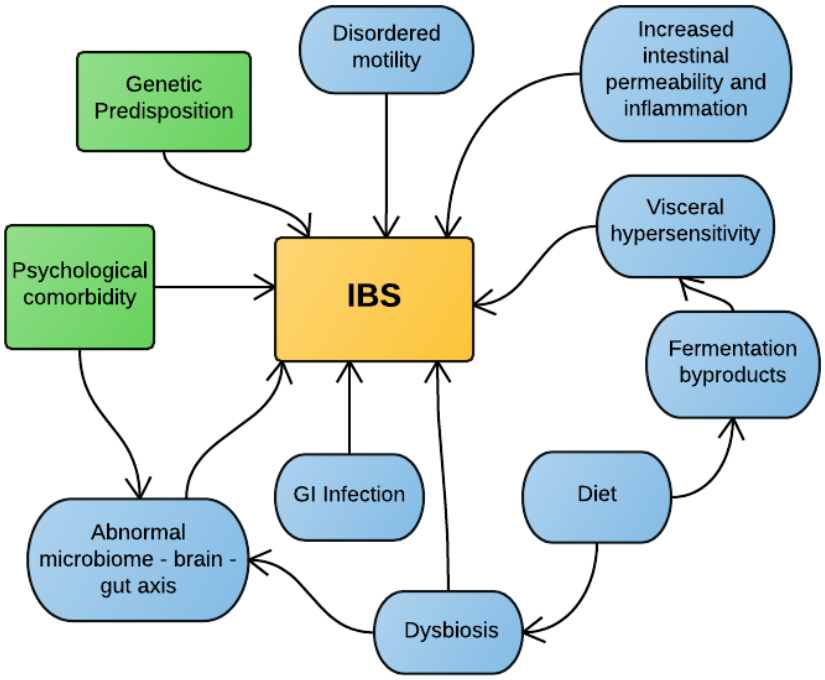

Figure 1.4 A summary of factors contributing to the pathogenesis of IBS

(Ladabaum et al., 2012) confirms the strong association between psychological comorbidity and IBS, although a clear cause-effect relationship is yet to be established.

Psychological distress may also play a secondary role in IBS. The presence of enhanced central activation when rectal distension is expected but not delivered compared with controls (Mayer et al., 2006) suggests hypervigilance exists in patients with IBS, which translates into a greater awareness of the presence of symptoms. This, combined with catastrophising (belief that the symptoms are due to serious disease), increases anxiety which subsequently worsens symptoms (Hungin et al., 2015). There is sufficient evidence to suggest that the central nervous system and psychological stress are directly and/or indirectly involved in the development of IBS, emphasising the importance of psychological therapy in at least a subset of patients.

1.1.5.2 Peripheral factors

The bulk of research relating to the pathogenesis of IBS relates to peripheral alterations including altered colonic motility, increased intestinal permeability, low grade inflammation, visceral hypersensitivity and dysbiosis of the GI microbiota.

1.1.5.2.1 Motility

Abnormal motility has historically been considered an important factor in the pathogenesis of IBS. Exaggerated motility response to stimuli such as food and stress has been demonstrated in the small intestine and the colon in patients with IBS (Gunnarsson and Simren, 2009), which may contribute to urgency, diarrhoea and pain symptoms. Slower colonic transit time is evident in IBS-C compared with IBS-D and IBS-M, and gastric emptying is delayed in 76% of patients according to a recent study employing a wireless motility capsule device technique (DuPont et al., 2014), although the relationship between gastric emptying measured by this technique and actual emptying time of a meal is uncertain. Colonic transit time in IBS-D has also been shown to be reduced compared with controls and delayed in IBS-C in a study measuring transit by scintigraphy after adjusting for body mass index (Manabe et al., 2010). Only 30% of each subtype group were clinically diagnosed with abnormal transit, however, which suggests motility abnormality may only contribute to IBS pathophysiology in a subset of patients. Altered serotonin mucosal secretion and/or uptake of serotonin into enterocytes are likely to be important in motility abnormalities in IBS (Spiller, 2007), and recent evidence confirms the key role of the microbiota in modulating colonic motility, at least in animal models (Kashyap et al., 2013).

1.1.5.2.2 Intestinal permeability and inflammation

Increased intestinal permeability (i.e. increased absorptive capacity of the epithelial layer) and impaired tight junction protein expression may lead to local GI dysfunction in up to 50% of patients with IBS (Ohman et al., 2015). It is proposed that this increased permeability leads to enhanced uptake of pathogenic bacteria or mediators of the commensal microbiota which leads to subsequent inflammatory changes (Ohman et al., 2015). Increased permeability has been associated with worse symptom profile (Zhou et al., 2009), however whether this alteration precedes the onset of IBS or occurs in response to the condition is unknown (Ohman et al., 2015).

There is accumulating evidence for the presence of low grade inflammation in some patients with IBS. Factors that support this theory include the increased risk of IBS following GI infection (post-infectious IBS, PI-IBS) (Marshall et al., 2010) and persistent increases in a range of mucosal inflammatory markers (Ohman and Simren, 2010). Increased blood concentrations of some (IL-6 and IL-8) but not all (activated T cells e.g. CD4+) inflammatory mediators, have also been demonstrated in IBS compared with healthy individuals (Matricon et al., 2012). The most consistent finding in this area is enhanced colonic infiltration of mucosal mast cells (Matricon et al., 2012), cells important for pathogen defence that may directly influence enteric sensory nerves (Barbara et al., 2004). This has also been demonstrated in the small intestine, but is most evident in the caecum and colon, with up to a 2-fold greater mast cell infiltration reported compared with controls (Matricon et al., 2012) and at levels which were comparable to samples of patients with ulcerative colitis in remission (Ahn et al., 2014).

An association between mast cell infiltration and symptoms in IBS has been demonstrated. One study reported a majority of patients with IBS (34/44, 76%) exhibited raised numbers of mast cells in colonic mucosal samples compared with controls (Barbara et al., 2004). Mast cell concentration in close proximity to sensory neurons was positively correlated with severity and frequency of abdominal pain, suggesting a direct influence of mast cells on symptom generation. Although this was not replicated in a recent large study in IBS-D (n=83) (Ahn et al., 2014), it might be that mast cell activation is more clinically relevant than cell number *per se* (Theoharides, 2014). This was evident in the former study, with raised mucosal histamine and tryptase in IBS samples compared with controls, a finding that has been replicated in other work (Matricon et al., 2012).

Taken together, it appears increased intestinal permeability and immune activation are important in a select subgroup of patients with IBS, however it is unclear whether these changes are a primary or secondary phenomenon. Many studies do not account for other factors that disrupt the mucosal barrier such as dietary exposure to food antigens (Fritscher-Ravens et al., 2014) or stress (Piche et al., 2008), and studies often do not differentiate PI-IBS from other subtypes. Clarification is still required regarding the relationship between intestinal permeability and/or low grade inflammation in IBS with symptom generation and the precise sites of the GI tract that are important in IBS.

1.1.5.2.3 Visceral hypersensitivity

It is proposed there is a dysregulation in the bidirectional signalling between the brain and GI tract in IBS. The microbiome-brain-gut axis refers to the relationship between these systems in the pathophysiology of FBD and other disorders. One key aspect of this dysregulation, and a major pathophysiological feature of IBS, is visceral hypersensitivity. This refers to the intensification of signals to the brain induced by luminal, mechanical (e.g. distension) and chemical stimuli in the GI tract, which leads to augmented symptom experience in the patient with IBS.

The most common test for measuring visceral hypersensitivity is rectal balloon distension. Forty years ago it was first demonstrated at least 50% of IBS patients have enhanced visceroperception on balloon inflation compared with only 6% of controls (Ritchie, 1973). Since then, studies demonstrate this is evident in IBS-D and IBS-C, is more common in females (Hungin et al., 2015), and is enhanced postprandially both in IBS and controls (Tornblom et al., 2014). Prevalence data varies which is in part due to differences in the criteria used to define hypersensitivity (Ludidi et al., 2012). Interestingly, there is also evidence of widespread hypersensitivity in IBS in response to thermal, ischemic and cold pressor stimuli, which may be secondary to persistent altered central processing in response to chronic nociceptive input from the GI tract (Zhou and Verne, 2011). Various rationales have been proposed for this enhanced sensitivity including altered TRVP1 expression, a receptor for noxious stimuli in rectal biopsies, aberrant central processing and the GI microbiota (Hungin et al., 2015).

1.1.5.2.4 GI microbiota and byproducts

The GI microbiota is becoming increasingly recognised as a key player in IBS. It may have direct influences at the mucosa and also via its luminal byproducts (e.g. gas, short chain fatty acids). Section 1.1.7 will focus in detail on the role of the GI microbiota in IBS.

1.1.6 The GI microbiota

The human GI tract harbors 10^{14} bacteria, 10 times more than the total number of cells in the human body and 150 times more genes than the human genome. In addition to bacteria, the GI system also harbors viruses, protozoa and fungi which likely all contribute to the overall ecosystem but contribute to only 1% of the genomic content (Qin et al., 2010). Low pH and fast transit inhibit growth of bacteria in the upper GI tract and bacterial density and diversity increases distally from the stomach with a final microbial concentration of approximately 10^{11}

cells/ml in the colon (Walter and Ley, 2011). The microbiota is a highly diverse, metabolically active community that exerts important influences on health and disease, and the host-microbiota relationship has been described as a mutualistic ecosystem, as both benefits from the relationship (Backhed et al., 2005). Two distinct GI microbiota populations exist: that within the colonic lumen and that within the mucosa overlying the GI epithelium (Zoetendal et al., 2002).

The luminal microbiota is likely a combination of nonadherent luminal bacteria and a mix of shed mucosal bacteria. There is significant variability in the composition of the luminal microbiota along the GI tract (Walter and Ley, 2011), suggesting that diet and environmental conditions have a powerful impact on this compartment. Conversely, the mucosal microbiota composition from the ileum to the rectum is highly stable within an individual (Lepage et al., 2005), suggesting a stronger host influence than environmental factors. Importantly, the mucosal microbiota are involved in 'crosstalk' at the mucosal border, between the lumen and the underlying tissue, where immune and enteroendocrine cells interact (Ohman et al., 2015).

1.1.6.1 Composition, function and association with health and disease

The composition of the microbiota has emerged as an important focus of research over recent decades in response to increasing understanding of its contribution to heath and disease. The two major phyla, Firmicutes and Bacteroidetes, comprise at least 90% of the known bacteria in the GI tract, and Actinobacteria contributes less than 10%. At the species level of taxonomy, the microbiota is characterised by a 'long-tail', with many species present in low abundance (Arumugam et al., 2011). Humans harbour approximately 160 bacterial species in total in the GI tract, 75 of which are found in up to 50% of individuals, indicating the presence of a 'core' microbiota (Qin et al., 2010). Despite the existence of a common core microbiota, large inter-individual variation is possible including in the abundance of the core species (Qin et al., 2010). Furthermore, there is an absence of detrimental bacteria in the human GI tract in healthy individuals, suggesting a distinction between a healthy microbiome and that in disease (The Human Microbiome Project Consortium, 2012).

A number of international groups have been established with the task of characterising and advancing our knowledge of the human microbiome, including MetaHIT (Metagenomics of the Human Intestinal Tract) in Europe and HMP (Human Microbiome Project) in the US. Early work from both consortiums suggested that healthy humans harbour one of three types of

microbiota clusters, termed 'enterotypes', driven by species composition (i.e. dominated by Bacteroides, Prevotella or Ruminoccous). It was postulated that each state may be prognostically and diagnostically predictive (Arumugam et al., 2011), however, the existence, and the number of distinct enterotype classifications has recently been questioned. In fact, it is postulated that these cluster classifications are driven more by environmental and host factors rather than inherent individual differences, and that interindividual microbiome differences are likely to be continuous rather than segregated. Furthermore, individual bacterial species may be more important for disease risk than enterotype classification (Knights et al., 2014).

The GI microbiota fulfils a number of diverse beneficial physiological functions. One key function is the breakdown of otherwise indigestible carbohydrates, leading to the production of short chain fatty acids (SCFA), which contribute to reduced colonic pH and inhibition of pathogens. Butyrate, one of the SCFA, has a number of important functions including provision of energy substrate to enterocytes and to some bacterial species, increasing expression of some epithelial tight junction proteins, and other immunomodulatory functions (Kannampalli et al., 2011). The GI microbiota also impacts on bile acid metabolism, synthesises a number of B vitamins and vitamin K, produces antimicrobial bacteriocins and is responsible for numerous other metabolic and immune functions. Although most of the recognised functions of the microbiota are beneficial, some of their metabolic outputs are harmful to the host e.g. the production of amines from protein catabolism that may react with nitrite can form carcinogenic nitrosamines (Montalto et al., 2009).

The GI microbiome may contribute to overall human health and disease. For example, one study demonstrated greater microbiota richness and diversity in an elderly cohort (n=178) was correlated with better nutritional status and health (Claesson et al., 2012), and studies in children suggest that a less diverse microbiota is associated with higher risk of allergic disease (Storro et al., 2013). Furthermore, some disease states (e.g. IBS, inflammatory bowel disease (IBD), and *Clostridium difficile*-associated disease) are characterised by low bacterial diversity (Lozupone et al., 2012), and a low gene count (reduced 'bacterial richness') is associated with greater overall adiposity and insulin resistance (Le Chatelier et al., 2013). Cause-effect relationships are not yet clear, but data from animal microbiota transplantation models suggests some of these changes are not merely a consequence of disease (Turnbaugh et al., 2006).

Together with the overall composition of the microbiota, specific bacteria are individually recognised for their health-promoting effects, some of which have been termed 'keystone species' (Scott et al., 2015). For example, *Faecalibacterium prausnitzii*, a member of the phylum Firmicutes, is one of the major commensal butyrate producers. It has been labelled as a biomarker of intestinal health in adults (Miquel et al., 2013) and is associated with maintenance of remission in IBD, although a specific role in IBS has not been identfiied. Bifidobacteria, a genus within the phylum Actinobacteria, has established beneficial effects on health. As well as fermenting carbohydrates and producing SCFA (acetic acid) and lactic acid, this group is immunomodulatory, may reduce induced colonic carcinogenesis in animals and has numerous other systemic effects including on blood cholesterol (Russell et al., 2011a). Conversely, a phylogenetic pattern of decreased *F. prausnitzii*, Bifidobacteria and *Akkermansia* spp. and increased Bacteroides is evident in low gene count individuals with an inflammatory phenotype (Le Chatelier et al., 2013), further supporting the potential importance of specific bacteria in disease pathogenesis. The absence of Bifidobacteria and enrichment of opportunistic bacteria from the phyla Proteobacteria and Spirochaetes in Hazda hunter-gatherers in Tanzania, however, suggests the concept of a 'healthy GI microbiota' may not be straightforward, that its functionality is more important, and that both are dependent on diet, the physical environment and other lifestyle factors (Schnorr et al., 2014).

1.1.6.2 Quantification

Stool or mucosal samples are used to quantify and/or characterise the colonic GI microbiota. Most studies in IBS have evaluated stool samples due to the ease and non-invasive nature of sampling, although it is recognised that the luminal microbiota is distinct from the mucosal compartment in IBS (Carroll et al., 2010). Culture-independent methods of microbiota analysis have led to an increase in the identification of bacterial species in the GI tract (Zoetendal et al., 2004) and enumeration of a majority of the GI microbiota (>70%) currently identified. Other recent advances in the study of the GI microbiota include the area of metagenomics, or the study of the overall microbiome (collective genomic material of the host microbiota). This has enabled characterisation of the functional capacity of the microbiota, which is essential for defining associations with disease, and is perhaps more meaningful than pure quantification. Metabolomic approaches, or techniques that study small molecule metabolites measurable in stool, urine and tissue produced by the microbiota and cells, have also been vital in contributing to our understanding of microbiota physiology and function. Specific GI microbiota quantification techniques are summarised in Chapter 2 (**Table 2.3**).

1.1.6.3 Factors affecting the microbiota

The GI microbiota community is shaped throughout life by a number of host-related and external factors. The human GI tract becomes colonised *in utero.* Early infant nutrition is known to be important modulator of microbiota composition and functioning, and may determine disease risk later life (Gritz et al., 2015). After infancy, children up to the age of four can be still clearly separated from adults based on their microbiota composition (Ringel-Kulka et al., 2013). Into adulthood, a more abundant and diverse microbiota community develops as the GI tract matures and through increased diet and environmental influence. Host factors such as gender, age (Claesson et al., 2012), ethnicity (Yatsunenko et al., 2012) and bodyweight (Ley et al., 2006) impact on the composition of the microbiota, some of which may also be explained by comorbidity, diet or drug exposure.

The GI microbiota of humans is relatively stable over time, however there are minor perturbations within this stable framework. The community is self-shaping as the microbiota 'assemble themselves according to available niches' (Walter and Ley, 2011), and compete for their position within the community, determined largely by the adaptability of the organism phenotype, the physical environmental condition of the GI tract (e.g. gastric acid, motility, GI secretions) (Jalanka-Tuovinen et al., 2011), genetic factors and colonisation history (Walter and Ley, 2011). There is an overall resilience of the healthy microbiome with some temporal variability, which enables the system to return to an equilibrium after minor shifts (Relman, 2012).

Of all external influences, antibiotics have the most pervasive effect on the structure and composition of the GI microbiota. For example, pyrosequencing analysis demonstrates that ciprofloxacin administration leads to a dramatic reduction in richness and diversity and a reduction in abundance of a third of taxa within 3-4 days. The alteration, as well as the rapidity and degree of return varied between individuals in this small study of 3 individuals (Dethlefsen et al., 2008). Other medications that impact on the GI microbiota, but not understood to have as an extreme impact, include proton pump inhibitors and prokinetics that are commonly prescribed in IBS (Simren et al., 2013). These are frequently not accounted for in randomised controlled trials (RCTs) that evaluate the effect of interventions on the microbiota but should be a consideration to prevent confounding effects.

1.1.6.3.1 Dietary impact on the GI microbiota

Two lines of evidence suggest that the GI microbiota is influenced by diet. Firstly, geographically distinct populations with vastly different habitual diets can be distinguished by their microbiome, and secondly, short term dietary interventions lead to clear alterations in microbiota composition.

A number of studies demonstrate clear distinctions in microbiota communities between individuals differing by habitual, long term diet. It is proposed that the microbiota is shaped over time according to habitual diet in order to extract appropriate nutrition from the food substrate provided (Schnorr et al., 2014). A recent study compared microbiota composition of African Americans (n=20) versus rural Africans (n=20) using DNA microarray. Dietary intakes differed in carbohydrate (47% of total energy vs 72%, respectively) and fibre content (14 g/d vs 66 g/d) based on 3-day dietary recalls. Stool-associated microbiota differed at the genus level, with the African American samples dominated by Bacteroides compared with the Prevotella-rich samples of the rural Africans (O'Keefe et al., 2015). This supports data from a previous study reporting profound differences in Prevotella-rich samples of African children compared with Italian children who followed a much lower fibre diet based on 3-day diet records (De Filippo et al., 2010). Along with genus level differences, higher diversity and richness of the microbiota in agrarian versus Western style communities is a common finding (Yatsunenko et al., 2012, Schnorr et al., 2014, De Filippo et al., 2010). Indeed, other studies suggest that divergence in microbiota composition in community-dwelling elderly individuals versus those in long term care (Claesson et al., 2012) and athletes versus bodyweight-matched controls (Clarke et al., 2014) is due to differences in habitual dietary intake. Some of these comparative studies also report alterations in microbiota byproducts (e.g. SCFA) (De Filippo et al., 2010, O'Keefe et al., 2015), indicating habitual diet may not only modulate the microbiota but also its functionality. However, there is little acknowledgement and/or agreement on the role played by host-specific and environmental factors (e.g. genotype, morbidity, sanitation) in influencing host physiology in these cross sectional studies.

Acute dietary interventions clearly have effects on the GI microbiota which most likely occur directly through altered substrate availability and/or indirectly through effects on transit time and pH. The addition of foodstuffs or nutrients such as cruciferous vegetable fibre (Li et al., 2009), polyphenols (Queipo-Ortuno et al., 2012), wholegrain wheat (Windey et al., 2014) and

corn (Holscher et al., 2015) have variable effects on the composition of the microbiota, and the effect of oats and barley have been investigated in *in vitro* studies.

Many studies examine the effect of manipulation of the whole diet on the GI microbiota, and in particular, the effect of extreme alteration to dietary carbohydrate or its contribution to energy intake (**Table 1.1**). Some studies reduce carbohydrate intake to as little 20 g/d (Duncan et al., 2007, Russell et al., 2011b, Brinkworth et al., 2009). Modification of the microbiota has also been reported in response to the gluten free diet (De Palma et al., 2009), which alters the dietary carbohydrate source and may unintentionally reduce carbohydrate intake. The most consistently reported changes in carbohydrate-reduced interventions include decreased abundance of the phylum Firmicutes, known to include many organisms capable of metabolising dietary plant polysaccharides, Bifidobacteria, and butyrate producers such as some Ruminococcaceae. Changes are rapid and can occur within 1-2 days (Wu et al., 2011, David et al., 2014). Studies evaluating the effect of short chain fermentable carbohydrate restriction on the GI microbiota are reviewed in Section 1.2.6.

There is clear evidence that diet-induced alterations in the microbiota are individually variable (Walker et al., 2011, Li et al., 2009), and in fact are markedly more variable than that induced by the intervention itself (Walker et al., 2011). This is probably determined by baseline differences in the microbiota and the compensatory capacity of the entire microbiota community. Indeed, baseline concentration of Bifidobacteria is positively correlated with the bifidogenic response associated with fibre supplementation (Whelan et al., 2005). This may be just one aspect of a more extensive phenomenon but similar findings in other bacterial groups have not yet been reported.

There are number of difficulties associated with researching the effect of dietary change on the microbiota. Firstly, there is the problem of collinearity, that is, changing one component of the diet leads to compensatory changes in other components e.g. a reduction in carbohydrate leads to increases in intake of protein and/or fat intake, which in itself might have specific effects on the microbiota. Secondly, dietary intake is not always precisely measured, which limits the confidence one can have that it is the intended dietary intervention *per se* that is effecting the microbiota alterations. Also, many studies include a maintenance diet pre-intervention, which may alter baseline microbiota composition and mask true response to the intervention, and many studies are crossover in design which brings the risk of carryover

Table 1.1 Summary of studies investigating the effect of carbohydrate modification on stool microbiota

Reference	Intervention	Duration	Participants	Design	Analysis method	Impact on microbiota vs baseline	Markers of fermentation vs. baseline
(David et al., 2014)	Animal rich (LC) vs plant rich(HC)	5 d	10 healthy	Randomised crossover	Sequencing	↑Bacteroides LC ↓Roseburia, ↓*R. bromii* LC	Various alterations in SCFA
(Fava et al., 2013)	HFLC vs HCLF	24 wk	88 at risk of metabolic syndrome	RCT	FISH	↓total bacteria HF/LC ↑Bifidobacteria, Bacteroides HC/LF ↑*F. prausnitzii* HC/LF (low GI)	No change in SCFA
(Wu et al., 2011)	HFLF vs LFHF	10 d	10 healthy	RCT	Shotgun metagenomics	Various taxa altered, individually variable responses	Not measured
(Walker et al., 2011)	Reduced energy HRS vs HNSP	10 wk	14 overweight men	Randomised crossover	qPCR and DGGE	↑*R. Bromii, %* Roseburia HRS ↑% Ruminococcus HRS	Not measured
(Russell et al., 2011b)	HPLC vs HPMC	4 wk	17 obese men	Randomised crossover	FISH	↓% Roseburia *E. Rectale* group HPLC, ↓% Bacteroides spp. HPLC	↓SCFA and ↑pH in HPLC
(Brinkworth et al., 2009)	Reduced energy HFLC vs HCLF	8 wk	91 overweight and obese	RCT	Culture	↓Bifidobacteria HF/LC	↓SCFA, no change in pH HFLC
(De Palma et al., 2009)	Gluten free	4 wk	10 healthy	Uncontrolled trial	FISH, flow cytometry, qPCR	↓Bifidobacteria, ↓*F. prausnitzii* ↓Lactobacillus	Not measured
(Duncan et al., 2007)	Reduced energy HPLC HPMC	4 wk	19 obese men	Randomised crossover	FISH	↓Roseburia *E. Rectale*, ↓Bifidobacteria, ↓ total bacteria HPLC HPMC	↓total SCFA HPMC, HPLC ↓butyrate HPLC
(Whelan et al., 2005)	Standard vs HF enteral formula	14 d	10 healthy	Randomised crossover	FISH	↓Clostridia, ↑Bifidobacteria HF	↓butyrate HF and standard

LC, low carbohydrate; HC, high carbohydrate; HFLC, high fat low carbohydrate; HCLF, high carbohydrate low fat; FISH, fluorescence *in situ* hybridisation; HFLF, high fat low fibre; LFHF, low fat high fibre; HRS, high resistant starch; HNSP, high non starch polysaccharide; HPLC, high protein low carbohydrate; high protein moderate carbohydrate; HF high fibre

effects. Finally, all of these studies are in healthy or obese patients and outcomes may not be representative of potential effects of dietary modulation in patients with IBS.

1.1.7 The GI microbiota in IBS

There is evidence from both animal and human studies to support the key role of the GI microbiota in the development and persistence of the IBS phenotype.

1.1.7.1 Proof of concept studies

Germfree mouse models provide direct evidence that the GI microbiota induce local gut dysfunction whilst controlling for factors such as diet. An elegant study recently demonstrated this using faecal transplantation. Human faecal microbiota from healthy volunteers or individuals with IBS was transferred to germfree mice and colonic physiology and function measured. Microbiota alteration was demonstrable at four weeks and maintained at seven weeks, with stool samples of IBS microbiota recipients generally mirroring their donors'. Features of IBS were also evident in these mice compared with mice inoculated with healthy microbiota, including visceral hypersensitivity and 2-3-fold increased 24-hour hydrogen gas production at seven weeks (Crouzet et al., 2013). Behavioural changes have also been identified in transplanted mice, suggesting dysbiosis might be responsible for behavioural symptoms as well as colonic motor dysfunction in IBS (Collins, 2014).

1.1.7.2 PI-IBS

There is clear evidence that GI bacterial infection leads to an increased likelihood of persistent functional GI symptoms despite clearance of the pathogen. The Walkerton Healthy study, the largest and longest study of PI-IBS, prospectively followed a cohort of 3900 individuals affected by a water-borne bacterial outbreak. Incidence of IBS in those who had had experienced acute gastroenteritis was 28% at two years after the outbreak (Marshall et al., 2006), which continued to remain higher than controls at eight years (15% vs 5%, respectively, odds ratio (OR) 3.1) (Marshall et al., 2010). Psychological morbidity, female gender, and the severity of the initial infection were identified as predisposing factors for persisting PI-IBS. Similar findings have been reported for incidence of IBS at six months after 'traveller's diarrhoea' (OR 3.51) in a recent systematic review and meta-analysis (Schwille-Kiuntke et al., 2015). Further work is required to evaluate the risk of IBS associated with individual pathogens, however these studies provide strong evidence that bacteria have a primary role in the onset of IBS in a subset of patients. Mechanisms underlying this process are unclear but may be via transient

alteration of the microbiota post infection, and ongoing dysbiosis in the presence of low grade mucosal inflammation (Collins, 2014).

1.1.7.3 Intestinal permeability and inflammation

A further line of evidence that supports the role of the microbiota in IBS pathogenesis relates to evidence of low grade immune activation in some patients. Dysbiosis is proposed to be one contributing factor for the enhanced expression of some toll-like receptors, degradation of epithelial tight junction proteins, increased intraepithelial permeability and dysregulation of the immune system and this is reviewed in detail elsewhere (Ohman et al., 2015). There is still much to understand about these observations in IBS, and in particular whether the role of the microbiota is aetiological or merely an epiphenomenon, and further studies that access the mucosa are required to enhance understanding of the microbiota neuroimmune 'crosstalk' at the mucosal border (Ohman et al., 2015).

1.1.7.4 Dysbiosis

A growing evidence base for dysbiosis in IBS suggests this might have a role in its pathogenesis. Differences in the luminal and mucosal GI microbiota of patients with IBS compared with controls have been reported at all levels of bacterial taxonomy using a range of qualitative and quantitative microbiological methods (**Table 1.2** and **Table 1.3**) and recent evidence suggests stool-associated microbiota in IBS is less similar to healthy controls than mucosal microbiota (Rangel et al., 2015). In regards to stool microbiota, decreases in Bifidobacteria, Bacteroidetes, and *F. prausnitzii*, and increases in Firmicutes, and the ratio of Firmicutes to Bacteroidetes are commonly reported. Two of four studies also demonstrate a reduction in mucosal Bifidobacteria compared with controls (Kerckhoffs et al., 2009, Parkes et al., 2012). As well as alterations in specific microbial taxa, reduced diversity and temporal instability are reported in IBS patients compared with controls (Jeffery et al., 2012, Carroll et al., 2010, Sundin et al., 2015, Matto et al., 2005), and individuals with functional GI symptoms exhibit a greater instability in the microbiota in response to dietary change (Manichanh et al., 2014).

There is a divergence in stool microbiota composition depending on IBS phenotype. For example, one study has shown higher abundance of stool Lactobacilli in IBS-D compared with IBS-C patients (Malinen et al., 2005). Furthermore, the microbiota of patients with PI-IBS has been reported to resemble IBS-D (Jalanka-Tuovinen et al., 2014), or, conversely, has been reported to be distinct from non PI-IBS (Sundin et al., 2015). Intriguingly, not all patients with

Table 1.2 Studies assessing stool microbiota composition in IBS

Reference	Participants	Method	IBS vs controls
(Sundin et al., 2015)	IBS n= 32 controls n=16	Phylogenetic 16S rRNA microarray	↑Firmicutes, ↑Clostridium Clusters IV & XIVa
(Rangel et al., 2015)	IBS n=33 controls n=16	Phylogenetic 16S rRNA microarray	↓Bacteroidetes, *F. prausnitzii*, ↑Actinobacteria, ↑Clostridium Cluster XIVa
(Jalanka-Tuovinen et al., 2014)	IBS n=23 controls n=11	qPCR, phylogenetic 16S rRNA microarray	↑ Bacteroidetes, ↑*R. Torques* ↓*Clostridiales,* methanogens
(Jeffery et al., 2012)	IBS n=37 controls n=20	pyrosequencing	↑Firmicutes: Bacteroidetes Clustering of normal-like and dysbiotic
(Carroll et al., 2012)	IBS-D n=23 controls n=23	qPCR, pyrosequencing	↓Enterobacteriaceae ↓*F. prausnitzii*
(Duboc et al., 2012)	IBS-D n=14 controls n=18	qPCR	↓Bifidobacteria ↑*E. coli*
(Rajilic-Stojanovic et al., 2011)	IBS n=62 controls n=46	qPCR, phylogenetic 16S rRNA microarray	↑Firmicutes: Bacteroides ↓Bacteroidetes , ↓Bifidobacteria, ↓*Faecalibacterium* spp.
(Ponnusamy et al., 2011)	IBS n=11 controls n=8	DGGE, qPCR	↑ Lactobacillus ↓Bifidobacteria
(Tana et al., 2010)	IBS n=26 controls n=26	Culture, qPCR	↑Veillonella, ↑Lactobacillus
(Codling et al., 2010)	IBS n=41 controls n=33	DGGE	↓diversity
(Carroll et al., 2010)	IBS-D n=10 controls n=10	Culture, qPCR	↓aerobic bacteria ↑Lactobacillus
(Krogius-Kurikka et al., 2009)	IBS-D n=10 controls n=23	G+C-profiling + sequencing of 16S rRNA genes	↑Proteobacteria, ↑Firmicutes, ↓Actinobacteria, ↓Bacteroidetes
(Kerckhoffs et al., 2009)	IBS n=41 controls n=26	FISH, PCR	↓Bifidobacterium, ↓% *B. catenulatum*
(Lyra et al., 2009)	IBS n=20 controls n=15	qPCR	↑*R. torques*, ↓*C. thermosuccinogenes*
(Kassinen et al., 2007)	IBS n=24 controls n=23	G+C-profiling + sequencing of 16S rRNA genes, qPCR	↓*Collinsella aerofaciens* ↑*C. cocleatum*
(Maukonen et al., 2006)	IBS n=16 controls n=16	PCR-DGGE	↓*C. coccoides* (IBS-C) ↓*Eubacterium rectale* (IBS-C)
(Malinen et al., 2005)	IBS n=27 controls n=22	qPCR	↓*Clostridium coccoides*, ↓*B. catenulatum,* ↓Lactobacillus (IBS-D vs IBS-C)
(Si et al., 2004)	IBS n=25 controls n=25	culture	↓ Bifidobacteria ↑Enterobacteriaceae
(Balsari et al., 1982)	IBS n=20 controls n=20	culture	↓Coliforms, ↓Lactobacillus, ↓Bifidobacteria

rRNA, ribosomal RNA ; qPCR, quantitative polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; FISH, fluorescence *in situ* hybridisation

Table 1.3 Studies assessing mucosal microbiota composition in IBS

Reference	Participants	Method	IBS vs controls
(Rangel et al., 2015)	IBS n=33 controls n=16	Phylogenetic 16S rRNA microarray	↓ Clostridiales I
(Parkes et al., 2012)	IBS n= 47 controls n=26	FISH	↑ total bacteria↑Bacteroides, ↑*E. rectale* ↑ *C. coccoides* cluster ↓ Bifidobacteria (IBS-D vs IBS-C)
(Carroll et al., 2010)	IBS-D n=10 controls n=10	Culture, qPCR	No difference
(Kerckhoffs et al., 2009)	IBS n=41 controls n=26	FISH, PCR (duodenal samples)	↓*B. catenulatum*

FISH, fluorescence *in situ* hybridisation; PCR, polymerase chain reaction

IBS have an altered microbiota. Those patients presenting with more adverse psychological traits have been reported to harbour a 'normal-like' microbiota composition that clusters separately to other IBS patients (Jeffery et al., 2012).

Moreover, there is recent evidence for the relationship of the microbiota with IBS symptoms. The most frequent finding is a negative relationship between stool Bifidobacteria concentration (Jalanka-Tuovinen et al., 2014, Rajilic-Stojanovic et al., 2011) and mucosal Bifidobacteria concentration (Parkes et al., 2012) and abdominal pain scores. Other findings include a positive relationship between abundance of *Ruminococcus torques*-like organisms (Jalanka-Tuovinen et al., 2014) with pain, a negative relationship between the abundance of *Proteobacteria* (Jeffery et al., 2012) with measures of pain, and a lower abundance of mucosal Bifidobacteria with greater stool frequency (Parkes et al., 2012). Alterations in microbiota composition in IBS have also been associated with depression. Specifically, a lower stool Firmicutes to Bacteroidetes ratio (Jeffery et al., 2012) and higher abundance of mucosal *E. Coli* (Parkes et al., 2012) is evident in those with higher anxiety and depression scores with IBS.

The nature of the clinical phenotype-microbiota relationship and whether dysbiosis in IBS is a primary or secondary phenomenon are still unclear. The association between dysbiosis and IBS symptoms is not consistent across studies; this may be due to variation in the IBS subtypes studied, differences in microbiota quantification techniques used, or in the degree of control over pre-study environmental factors that might influence the microbiota (e.g. antibiotics, diet). Precision of patient characterisation also varies significantly between studies, and given the heterogeneous nature of IBS, is an important consideration for future work in this area.

1.1.7.5 Microbiota byproducts

Luminal bacterial metabolic byproducts may generate symptoms in IBS. The SCFA butyrate dose-dependently induces visceral hypersensitivity in mice (Bourdu et al., 2005) and, indeed, stool acetic and propionic acid concentrations are higher in IBS and have been associated with higher symptom scores (Tana et al., 2010). A recent study detected no difference in stool SCFA between patients with IBS and controls, although there was a lower colonic pH, suggesting greater colonic fermentation and SCFA production in patients with IBS (Ringel-Kulka et al., 2015). Interestingly, there was a negative correlation between SCFA and colonic transit time and a positive correlation between colonic pH and colonic transit time, suggesting an aetiological role for fermentation byproducts in determining motility and possibly symptoms in IBS (Ringel-Kulka et al., 2015). In contrast, however, butyrate has been shown to dose dependently improve visceral hypersensitivity in healthy individuals (Vanhoutvin et al., 2009), and therefore the effects of SCFA on IBS symptoms require further clarification whilst controlling for diet.

Fermentative breakdown of food substrates by the microbiota also generates hydrogen, carbon dioxide, methane and hydrogen sulphide gas, which are of significance in IBS. Intestinal hydrogen production from fermentation is the only source of hydrogen production in humans, rendering it a useful proxy for fermentation capacity. Endogenous fermentative gases are disposed of via 3 routes 1) absorption into the circulation, 2) disposal by gas-consuming bacteria (e.g. acetogenic bacteria convert carbon dioxide and hydrogen into acetic acid and water) and 3) elimination per rectum. Animal work suggests microbiota from patients with IBS induces a marked two to three-fold increase in hydrogen production compared with controls (Crouzet et al., 2013). Conversely, diet-controlled (King et al., 1998) and diet-uncontrolled human studies (Tana et al., 2010) suggest individuals with IBS do not produce more hydrogen than controls although the rate of hydrogen production may be altered and influenced by diet (King et al., 1998), and may lead to lower total gas produced compared with a standard diet (Dear et al., 2005). Importantly, colonic gas volume correlates with peak symptom intensity in response to dietary challenge (Major et al., 2015b), suggesting a direct effect of fermentative gas on symptoms, and underlining the potential benefit of a reduction in dietary fermentable substrates.

Gas transit may also be important in determining symptoms in IBS. An elegant scintigraphy study demonstrated the relevance of this by examining the colonic response to a one-hour gas

infusion in patients with IBS and bloating. Gas clearance from the proximal colon was impaired at 30 minutes compared with controls, and this was accompanied by exacerbation of GI symptoms (Hernando-Harder et al., 2010).

There has been interest in investigating the association between the hydrogen-disposing bacterial groups and IBS. Some evidence for higher concentrations of methanogens in IBS-C exists, but the role of the microbiota in influencing gas volume in other IBS subtypes is unclear (Rajilic-Stojanovic et al., 2015). Intestinal gas homeostasis is complex and not completely understood, but is likely the product of many independent factors, including the gas disposal pathways and microbiota composition. Dietary substrate availability is clearly important and presents an opportunity for mediating symptom provocation.

1.1.7.6 Microbiota-directed therapy

Microbiota-directed interventions in IBS elicit improvements in GI symptoms in some studies, which provide further evidence of the role of the microbiota in IBS pathogenesis. The therapeutic role of these interventions in IBS is discussed in Section 1.3.

1.1.8 Treatment options in IBS

There are numerous pathophysiological mechanisms underlying the development of IBS and more research is required to confirm the contribution of each across IBS subtypes. Understanding the aetiology and the disease course of IBS is important in order to devise optimal treatment regimens. The following section summarises the current treatment options available for patients with IBS.

1.1.8.1 Medical therapy

The complex pathophysiology of IBS, symptom heterogeneity of presenting patients and instability of symptoms raises treatment challenges. Treatment is largely empirical and after lifestyle considerations (stress reduction, exercise, diet) have been addressed, medical treatment is targeted towards the predominant symptom with antispasmodics, anti-diarrhoeals or over-the-counter non gas producing laxatives (osmotic, bulking-forming or stool softeners) with an emphasis on self-management (NICE, 2015). There is little recent efficacy data of these first line treatment options in IBS. One systematic review and meta-analysis reported greater effectiveness of antispasmodics over placebo in IBS, although there was significant heterogeneity between studies, publication bias, greater adverse events reported

versus placebo, and a majority of the medications included were not available in the UK (Ford et al., 2008). Furthermore, studies included in the analysis recruited patients from all levels of medical care, which may mask true treatment effect in specific patient subgroups.

If first line therapies have not been effective, low-dose antidepressants (tricyclic antidepressants or selective serotonin reuptake inhibitors) are effective in some patients. New agents with acceptable safety profiles are also available in secondary and tertiary care. For example, lubiprostone and linaclotide that increase colonic motility have demonstrated efficacy in patients with constipation, and serotonin antagonists (e.g ondansetron) may be effective in IBS-D. Psychological and behavioural interventions, including hypnotherapy, may also benefit some patients (NICE, 2015), however access to services offering these treatments may be limited.

1.1.8.2 Antibiotic therapy

It is plausible that if dysbiosis is a major aetiological factor in IBS, interventions that target the microbiota should be effective in some patients. One way of potentially restoring an abnormal microbiota is through antibiotic therapy. Rifaximin, a non-absorbable antibiotic that has received the most attention in the treatment of IBS, was recently the subject of a meta-analysis. Therapeutic benefit was reported for adequate relief of global symptoms (OR 1.57), equivalent to a number needed to treat (NNT) of 10.2 (Menees et al., 2012), and it had effects on improving bloating compared with placebo (OR 1.55). Overall frequency of adverse events was similar between groups, including for diarrhoea, suggesting this may be an effective treatment for some IBS patients, although efficacy and safety profile beyond four months is still to be established.

Antibiotics reduce overall GI microbiota load in the colon, and thereby reduce colonic gas production (Dear et al., 2005). Other effects are largely unknown, although a series of studies in animal models of IBS suggests rifaximin not only reduces bacterial content by up to 84%, but has specific effects at the level of the colonic mucosa, improving integrity and reducing inflammation, and normalising visceral hypersensitivity. Interestingly, a consistent finding in these studies is an increased abundance in ileal Lactobacilli in the animals receiving rifaximin, which is hypothesised to be responsible for the anti-inflammatory effect (Gao et al., 2014).

Overall, antibiotics appear to be an effective intervention in a proportion of patients. However, they may also increase the risk of developing GI symptoms (Maxwell et al., 2002). The paradox that antibiotics are used as a treatment but also are involved in the pathogenesis of functional GI symptoms is difficult to resolve, and the role of antibiotics in the pathogenesis of IBS may be related to a lower threshold for medical consultation (Whitehead et al., 2007), which leads to a greater likelihood of antibotic therapy in these patients. More data are required to understand the action of antibiotics in IBS, define predictors of response, clarify optimum dose, confirm durability of effectiveness, and assess long term safety.

1.1.8.3 Diet

Many patients believe that their IBS symptoms are related to diet. There is generally a lack of evidence regarding the underlying mechanisms by which food provokes symptoms in IBS, which has limited the development of validated diagnostic tests to identify specific food triggers. Furthermore, evidence for the effect of dietary intervention on IBS symptoms has historically been scarce. Data regarding manipulation of dietary fibre intake in IBS is inconsistent (Eswaran et al., 2013), uncontrolled trials of exclusion diets followed by reintroduction indicate individual foods (e.g. wheat) exacerbate symptoms (Nanda et al., 1989, Parker et al., 2008), but mechanisms by which they cause symptoms have not been identified. Furthermore, although associations between IBS symptoms and intake of caffeine, alcohol and fat have been reported in cross-sectional studies, no RCTs investigating the effect of their restriction have been performed. Nevertheless, interest in the dietary management of IBS continues to grow amongst clinicians and patients. In particular, a diet low in short chain fermentable carbohydrates (the low FODMAP diet) has gained significant attention. Dietary modification of the GI microbiota through probiotics or prebiotics present other potential approaches for the management of IBS. Each of these three strategies will be reviewed (Sections 1.2, 1.3).

1.2 The low FODMAP diet

1.2.1 Introduction

Carbohydrates are an important component of the diet in humans and in UK adults they contribute to nearly half of total energy intake. They are a diverse group of substances characterised by a range of physical and physiological properties and have important roles in energy metabolism and colonic function.

Restriction of individual carbohydrates (e.g. lactose, fructose) has been considered for the management of GI symptoms for many years. Recently, broader restriction of several short chain fermentable carbohydrates has been of clinical and research interest. This diet has been termed a low 'fermentable oligosaccharides, disaccharides, monosaccharides and polyols' (FODMAP) diet. Section 1.2 will describe the individual components of this group of fermentable carbohydrates, evaluate the literature regarding the mechanisms underlying a low FODMAP diet and the available evidence regarding its clinical effectiveness in IBS, and discuss its impact on the GI microbiota and nutrient intake.

1.2.2 Fermentable carbohydrates

Some dietary carbohydrates such as glucose, sucrose and starch, are completely digested and absorbed in the small intestine. Carbohydrates known to be indigestible in humans include non starch polysaccharides (NSP), resistant starch, short chain carbohydrates and some polyols (Elia and Cummings, 2007). Up to 40 g of unabsorbed carbohydrate enters the colon per day in individuals consuming a Western diet (Scott et al., 2013). This then becomes available for bacterial fermentation either due to the absence, or reduced concentration, of suitable hydrolase enzymes for digestion, or in the case of some disaccharides and monosaccharides, due to incomplete absorption in the small intestine. The degree of carbohydrate digestibility is further influenced by the presence of disease (e.g. malabsorption disorders), interindividual variation, and in some cases, transit time and the dose consumed (Elia and Cummings, 2007).

On entering the colon, carbohydrates with a high degree of polymerisation (DP>10) are fermented more slowly and produce less gas than their counterpart short chain carbohydrates (DP<10) (Hernot et al., 2009). Long chain polysaccharides contribute to a substantial proportion of indigestible dietary carbohydrate, and include plant cell wall NSP (e.g. cellulose, hemicelluloses and pectin), psyllium and resistant starch. Along with these long chain carbohydrates, smaller quantities of protein and fat also enter the colon from exogenous (dietary) and endogenous (e.g. red blood cells, sloughed epithelial cells) sources. Their effect on fermentation and metabolic byproducts is less well studied (Scott et al., 2013). The remaining chapter will review the short chain carbohydrates (i.e. fructans, galacto-oligosaccharides, lactose, fructose and polyols) and review their dietary sources, digestibility and absorption, and physiological effects in the GI tract.

1.2.2.1 Fructans

Fructans, also termed 'inulin-type fructans' are a major dietary source of fermentable carbohydrates. They are either linear or branched fructose oligosaccharides that include inulin, oligofructose and fructo-oligosaccharides (FOS), and have a DP of 2-60. There is minimal digestion of fructans in the small intestine due to the lack of enzymes in the human GI tract able to hydrolyse the β (2-1) fructosyl-fructose glycosidic bonds. Up to 90% of fructans survive the small intestine undigested (Barrett et al., 2010, Hernot et al., 2009) and as a result of their unique properties in the colon they are classified as prebiotics (Section 1.3.1).

Fructans are present as storage carbohydrates in plant products (**Table 1.4**). A majority of dietary fructans come from wheat, onion, garlic and leek, which are relatively low in fructans but consumed in large quantities (van Loo, 1995, Dunn et al., 2011). Commercial fructans derived from sucrose or chicory root are increasingly added to pre-prepared foods due to their textural and sensory properties and potential health benefits, including their low energy value. They are commercially added to low fat yoghurt, ice cream, breakfast cereals, protein powders, multivitamin and mineral products and probiotic supplements. Average fructan intake in healthy individuals is reported to be up to 4 g/d (Dunn et al., 2011) and slightly lower in patients with IBS at 2-3.5 g/d (Staudacher et al., 2012, Bohn et al., 2015).

Table 1.4 FODMAP content of selected foods

FODMAP	Example dietary source	FODMAP content g/100g	Reference
Fructans	Jerusalem Artichoke tuber	19-25	(van Loo, 1995)
	All Bran®	2.4	(Biesiekierski et al., 2011)
	Bread, rye	1.9	(Whelan et al., 2011)
GOS	Split peas, boiled	1.9	(Biesiekierski et al., 2011)
	Beans, borlotti	1.0	(Biesiekierski et al., 2011)
	Beans, soya	0.8	(Biesiekierski et al., 2011)
Lactose	Milk, sheep's	5.1	(Food Standards Agency, 2002)
	Milk, cow's	4.6	(Food Standards Agency, 2002)
	Milk, goat's	4.4	(Food Standards Agency, 2002)
Fructose	Honey	41.8	(Food Standards Agency, 2002)
	Currants	33.3	(Food Standards Agency, 2002)
	Apple	5.9	(Food Standards Agency, 2002)
Polyols	Sugar free gum	41.9	(Yao et al., 2014)
	Plum	2.4	(Yao et al., 2014)
	Celery	1.5	(Yao et al., 2014)

GOS, galacto-oligosaccharides

As well as their prebiotic properties, fructans have other beneficial physiological effects. This includes bulking of stool through increasing stool biomass, and reducing the risk of colon cancer in animal models, which is proposed to be associated with increased butyrate production (Roberfroid, 2007). There is also evidence for beneficial effects systemically such as appetite regulation, lipid metabolism, and improved calcium bioavailability (Roberfroid, 2007). However, despite the beneficial effects on health, acute modest doses (7 g) may induce GI symptoms in IBS (Silk et al., 2009) and in healthy individuals (Bonnema et al., 2010), although isolated fructan restriction in IBS has not been evaluated.

1.2.2.2 Galacto-oligosaccharides (GOS)

Galacto-oligosaccharides (GOS) consist of galactose monomers (DP<10) with a terminal glucose unit, and include raffinose, stachyose and verbascose. Humans lack the α-galactosidase enzyme, which results in the availability of GOS for colonic fermentation and its prebiotic effect (Section 1.3.1). Although overall food composition data for GOS is more sparse than for fructans, identified naturally occurring sources of GOS include human breast milk, pulses, legumes and some grains, nuts and seeds (Biesiekierski et al., 2011, Cummings and Stephen, 2007) (**Table 1.4**). GOS can also be commercially produced via β-galactosidase enzymatic treatment of lactose, and is commonly added to infant formula, dairy products and beverages. Dietary intake data in healthy individuals is not available, however intake in individuals with IBS is low at 0.5-2 g/d (Staudacher et al., 2012, Bohn et al., 2015).

Despite their prebiotic effect, studies in healthy individuals indicate relatively small doses of GOS induce GI symptoms. For example, acute ingestion of 80 g conventional soya flour (3 g GOS) elicits greater frequency of flatus compared with low stachyose and raffinose soya flour (0.5 g GOS) in healthy individuals (Suarez et al., 1999). Galactosidase enzyme supplementation reduces symptom score after high GOS test meals (7.5 g GOS) in healthy individuals compared with placebo, confirming this is a GOS-specific effect (Di Stefano et al., 2007), however isolated GOS restriction in IBS has not been investigated.

1.2.2.3 Lactose

Lactose is a disaccharide of glucose and galactose. Lactase hydrolysis of lactose into its constituent monosaccharides is required for absorption, and up to 70% of humans exhibit hypolactasia which results in lactose malabsorption (Lomer et al., 2008). The reported

prevalence of lactose malabsorption in IBS is variable, but three recent studies of patients with GI symptoms referred for hydrogen breath testing report a prevalence of 20-30%, which is not different to healthy individuals (Barrett et al., 2009, Wilder-Smith et al., 2013, Bate et al., 2010) although prevalence is much higher in Asian, African, and South American populations. Diagnosis of lactose malabsorption is not clinically meaningful unless lactose consumption exacerbates GI symptoms i.e. lactose intolerance.

Lactose malabsorption can be measured in a variety of ways including measurement of breath hydrogen and methane in response to lactose challenge (lactose breath test), blood glucose concentration in response to a lactose challenge (lactose tolerance test), and evaluation of lactase activity in jejunal biopsy samples. There are a variety of limitations associated with these methods, including the time burden, lack of agreement on lactose dose for breath and tolerance tests, and the invasiveness of measuring lactase activity. There has been considerable debate regarding the usefulness of breath testing for the identification of tolerance to lactose in IBS. Presence of lactose malabsorption on breath testing is not representative of intolerance to lactose, likely due to interindividual differences in visceral hypersensitivity (Yang et al., 2013). Lack of agreement on breath test methodology and poor access to breath test facilities limits its use. Genotyping is useful as an objective diagnostic tool, whereas simple dietary restriction and rechallenge may be more accessible for most patients.

Lactose is naturally present in mammalian milk (e.g. cow's, goat's and sheep's), and products derived from it (e.g. yoghurt, ice cream, cheese) (**Table 1.4**). It is commercially added to baked goods as a browning agent or humectant (Lomer et al., 2008). Average intake of lactose is reported to be 12 g/d in the healthy population and 7-10 g/d in IBS (Staudacher et al., 2012, Bohn et al., 2015). A total dose of 12 g/d has been suggested as tolerable in those with lactose intolerance, meaning complete restriction is not required (Lomer et al., 2008). Lactose-reduced or hydrolysed lactose products (e.g. lactose free milk or yoghurt) are popular and convenient substitutes for standard lactose-containing products.

Despite studies reporting that large doses of lactose solution (20-50 g) lead to GI symptoms in IBS (Wilder-Smith et al., 2013, Zhu et al., 2013) only a small number of RCTs and observational studies have been conducted investigating lactose restriction, with variable responses demonstrated. In one of the largest RCTs to date (n=122), lactose restriction was effective in

improving symptoms of only 40% of patients with IBS with confirmed lactose malabsorption on breath testing, despite those positive for lactose malabsorption experiencing greater symptoms during the test compared with those without lactose malabsorption (Parker et al., 2001). Furthermore, of those who subsequently improved on a low lactose diet, double-blind placebo-controlled capsule challenge was positive in only 29%. Therefore, although lactose restriction may be helpful in a small proportion of patients with lactose intolerance, it may represent response to other aspects of the dietary restriction or to placebo effect, suggesting overall lactose restriction in isolation is relatively ineffective in IBS.

1.2.2.4 Fructose

Fructose is a 6-carbon monosaccharide that is dose-dependently and variably absorbed (Jones et al., 2011). Fructose absorption can occur through a number of routes of facilitated transport. The most widely researched are via the fructose-specific GLUT5 transporter and the GLUT2 transporter on the apical membrane of the intestinal epithelium, the latter involving a process of glucose-fructose co-transport. A third transporter (GLUT7) has also been identified, but is unlikely to be a major candidate due to its distal location in the ileum (Jones et al., 2011). There is considerable debate about the distribution and role of these transporters in fructose absorption. It is clear, however, that a fructose-glucose ratio of 1:1 dramatically improves fructose absorption (Truswell et al., 1988).

Reported prevalence of fructose malabsorption, or the incomplete absorption of fructose, varies significantly between studies most likely due to variations in breath testing methodology. However, two large studies using identical challenge doses (35 g) and diagnostic criteria reported 25-55% of healthy people and a similar proportion of those with IBS present with fructose malabsorption (Barrett et al., 2009, Bate et al., 2010). Therefore, it appears evidence of fructose malabsorption without symptoms is a normal phenomenon, and, as is the case with lactose, it is only clinically relevant if symptoms are present during testing.

Major dietary sources of fructose in the US include fruit, fruit products and products sweetened with high-fructose sweeteners (Marriott et al., 2009) (**Table 1.4**). Overall average daily fructose intake in the general US population has been reported as 41 g/d' (Marriott et al., 2009). Although fructose intake in the healthy UK population has not been reported, much lower intakes have been reported in IBS patients in the UK and Europe (14-17 g/d) compared with the US (Staudacher et al., 2012, Bohn et al., 2015). It is clear that an acute 20-35 g

fructose load induce symptoms in some patients with IBS (Shepherd et al., 2008, Wilder-Smith et al., 2013), which is not always associated with evidence of malabsorption on breath testing (Wilder-Smith et al., 2013). The evidence for isolated dietary fructose restriction in IBS is unclear, as studies are observational and uncontrolled in nature (Ledochowski et al., 2000, Born et al., 2006).

1.2.2.5 Polyols

Polyols are sugar alcohols such as the monosaccharides sorbitol and mannitol. Their absorption is passive, variable between individuals and affected both by molecular size and organic disease (Fordtran et al., 1967, Elia and Cummings, 2007). The inability to absorb a complete 10 g dose of sorbitol has been reported in 60-70% of healthy individuals (Hyams, 1983) and patients with IBS (Yao et al., 2014). There is greater absorption of mannitol than sorbitol in IBS, a finding that is hypothesised to be due to its differing hydroxyl position or to luminal factors affecting its water solubility and therefore absorption (Yao et al., 2014). Polyols are potential candidate prebiotics as they are available for fermentation, however evidence for their ability to selectively stimulate specific microbiota is limited.

Fruit and vegetables are natural sources of sorbitol and mannitol, and sugar-free chewing gum is a significant source, containing at least 10 times the sorbitol per gram compared with many fruit and vegetables (Yao et al., 2014) (**Table 1.4**). Other polyols include the monosaccharides xylitol, erythritol, and the disaccharides isomalt and maltitol, many of which are commonly used as bulk sweetening agents in the food industry due to their sweetness, mouth feel, temperature stability and low calorific value. Polyol intakes are not well documented, but have been reported at $\leq$1 g/d in IBS (Staudacher et al., 2012, Bohn et al., 2015).

Acute loads of sorbitol (10 g) or mannitol (10-17 g) in IBS clearly induce GI symptoms (Marciani et al., 2010, Yao et al., 2014). Furthermore, symptom improvement on sorbitol restriction has been demonstrated in 50-70% of patients with FBD (Fernandez-Banares et al., 2006, Goldstein et al., 2000, Born et al., 2006). However, a majority of these studies also restrict other carbohydrates (e.g. fructose, lactose), are observational in nature, and do not clearly define symptom response. Therefore whether isolated polyol restriction in IBS is effective is unclear.

1.2.3 GI effects of FODMAPs

There is considerable evidence that acute challenge with individual FODMAP carbohydrates induces GI symptoms in IBS, as described above. The dose-dependent and cumulative effect of FODMAPs been demonstrated using individual FODMAPs in solution (Rumessen and Gudmand-Hoyer, 1988, Shepherd et al., 2008), although only one study has tightly controlled for background diet by providing all food and fluid for the duration of the study (Shepherd et al., 2008). Increasing FODMAP intake through consumption of high FODMAP foods also leads to increased GI symptoms in individuals with excessive flatulence (Manichanh et al., 2014) and in patients with IBS (Ong et al., 2010) within a short period of time (2-3 days). The physiological effects of FODMAPs in the GI tract that are proposed to induce GI symptoms will now be described and are depicted in **Figure 1.5** and a summary of relevant studies is presented in **Table 1.5**.

1.2.3.1 Small intestinal water

Certain FODMAPs increase small intestinal water. This has been demonstrated by ileostomy recovery and magnetic resonance imaging (MRI) studies. Indeed, one randomised single blind crossover feeding study in 10 patients with quiescent IBD showed effluent water increased by 20% after a 4-day high FODMAP diet (112 g FODMAPs/d) compared with a diet with very low FODMAP content (6 g FODMAPs/d) (Barrett et al., 2010) (**Table 1.5**). An even greater effect on small intestinal water has been demonstrated in response to acute challenge using MRI. Healthy individuals were found to have a 10-fold higher small intestinal water volume 40 minutes after consumption of a 17.5 g mannitol solution compared with an equimolar glucose solution (Marciani et al., 2010), and significant rises also occur after administration of 40 g fructose (Murray et al., 2014, Major et al., 2015b), which is partially resolved through contemporaneous ingestion of 40 g glucose (Murray et al., 2014). Inulin, conversely, has no effect on small intestinal water compared with glucose (Murray et al., 2014, Major et al., 2015b), however further study is needed on the effect of smaller DP fructans that are more representative of those found in the diet.

Increased small intestinal water volume, particularly in the context of visceral hypersensitivity in IBS, might provoke abdominal pain and bloating, and in the absence of adaptive colonic water absorption might result in diarrhoea. Indeed, the enhanced small intestinal water associated with intake of FODMAPs has recently been correlated with symptom exacerbation

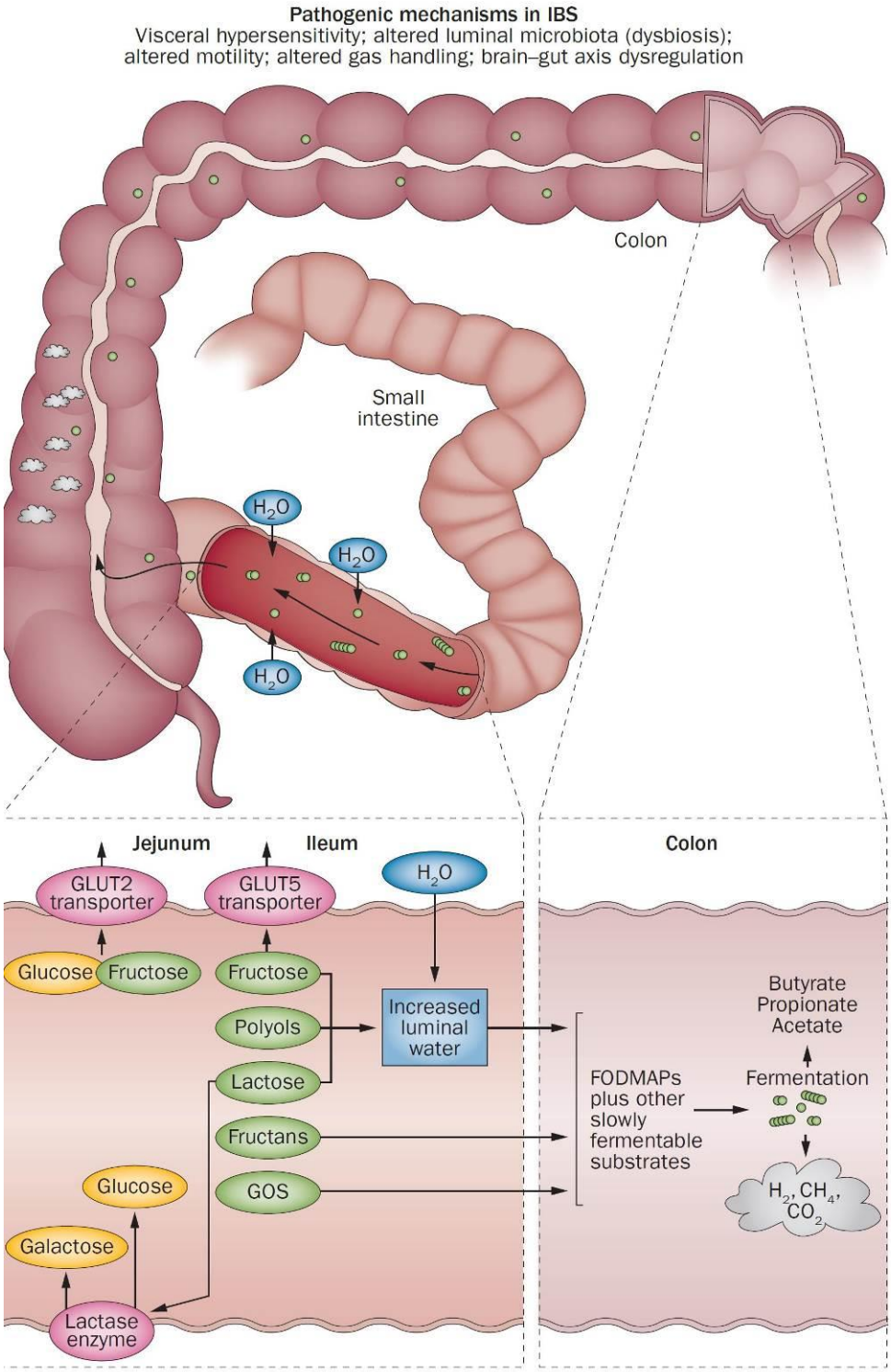

Figure 1.5 Mechanisms by which FODMAPs might induce symptoms in IBS. Unabsorbed fructose, polyols and lactose lead to water shifts in the ileum. Unabsorbed FODMAPs are fermented in the colon leading to luminal gas production. In the setting of visceral hypersensitivity and altered colonic functioning the resulting luminal distension leads to symptom exacerbation (Staudacher et al., 2014)

Table 1.5 Studies investigating the effect of FODMAPs on small intestinal water and colonic fermentation

Reference	Study design	Participants	Intervention	Outcome measures	Findings
Small intestinal water					
(Major et al., 2015b)	Randomised, double blind crossover	IBS n=29	40 g glucose solution (control) 40 g fructose solution 40 g inulin solution	SBWC using MRI	↑SBWC fructose vs glucose ($p<0.005$) Fructose correlation SBWC with symptoms ($p<0.05$)
(Murray et al., 2014)	Randomised, single blind, crossover	Healthy n=16	40 g glucose solution (control) 40 g fructose solution 40 g inulin solution 40 g fructose + 40g glucose solution	SBWC using MRI	↑SBWC fructose vs glucose (mean difference 25 l/min; $p<0.005$) ↓SBWC fructose + glucose (mean difference 16 l/min) vs fructose (not sig) SBWC inulin no effect vs glucose ($p>0.7$)
(Barrett et al., 2010)	Randomised, single blind, crossover	Patients with quiescent IBD and ileostomy n=10	4-day high FODMAP diet (112 g/d) 4-day low FODMAP diet (6 g/d)	Effluent weight Effluent water content	↑effluent weight high vs low FODMAP diet (409 g vs 504 g; $p=0.01$) ↑water content high vs low FODMAP diet (20% increase; $p=0.013$)
(Marciani et al., 2010)	Randomised, single blind, crossover	Healthy n=11	17.5 g glucose solution (control) 17.5 g mannitol solution	SBWC using MRI	↑SBWC mannitol vs glucose at 40 minutes (381 ml vs 47 ml; $p<0.001$)
Fermentation					
(Major et al., 2015b)	Randomised, double blind crossover	IBS n=29	40 g glucose solution (control) 40 g fructose solution 40 g inulin solution	Breath H_2 samples over 300 min Colonic volume using MRI	↑colonic gas and volume inulin vs fructose, glucose ($p<0.05$) Inulin correlation colonic gas with symptoms ($p<0.05$)
(Murray et al., 2014)	Randomised, single blind, crossover	Healthy n=16	40 g glucose solution (control) 40 g fructose solution 40 g inulin solution 40 g fructose + 40g glucose solution	Breath H_2 samples over 400min	↑H_2 inulin vs glucose ($p<0.0001$) and fructose ($p<0.05$) ↑colonic gas inulin vs glucose ($p<0.05$)
(Ong et al., 2010)	Randomised, single blind, crossover	IBS n=15 Healthy n=15	2-day high FODMAP diet (50 g/d) 2-day low FODMAP diet (9 g/d)	Breath H_2 14 hours on day 2	↑H_2 production in high vs low FODMAP diet in both IBS (242 ppm vs 62 ppm; $p<0.001$) and controls (181 ppm vs 43 ppm; $p<0.001$)

SBWC, small bowel water content; MRI, magnetic resonance imaging; H_2, hydrogen

in patients with IBS (Major et al., 2015b), suggesting for the first time that there is a relationship between the increased small intestinal water and GI symptoms. Interestingly, small intestinal water content was higher even in those that were asymptomatic, signifying enhanced visceral hypersensitivity in the symptomatic group may have been central to provocation of symptoms.

1.2.3.2 Fermentation

The availability of short chain carbohydrates for colonic fermentation leads to increased hydrogen and methane production, resulting in luminal distension and pain in IBS (**Table 1.5**). The first study to demonstrate this was a controlled, crossover feeding study in patients with IBS (n=15) and healthy individuals (n=15). A high FODMAP diet (50 g/d) led to a marked increase in breath hydrogen production compared with a low FODMAP diet (<10 g/d) in patients with IBS and in healthy individuals (Ong et al., 2010). Furthermore, individual FODMAPs elicit distinct hydrogen responses. Hydrogen release occurs later, remains elevated for longer and is significantly greater after 40 g inulin compared with fructose in healthy individuals (Murray et al., 2014). Direct measurements of colonic gas volume using MRI also demonstrated substantially greater peak colonic volume after inulin versus fructose (265 vs 142 volume/ml) (Major et al., 2015b). This is likely due to differences in transit time, which leads to variable availability for fermentation in the proximal colon, and variable fermentation rate between carbohydrates of different molecular geometry (Hernot et al., 2009).

1.2.3.3 Motility, colonic volume and microbiota

Some preliminary evidence suggests there are other means by which FODMAPs might induce symptoms. Firstly, some FODMAPs increase GI motility. Small intestinal transit time is increased following ingestion of 30 g of a fructose-sorbitol mixture in healthy individuals (Madsen et al., 2006), which further reduces availability of these substrates for absorption, increasing their exposure to bacteria for fermentation. Secondly, a short term low FODMAP diet appears to influence colonic volume measured on MRI. Colonic volume in healthy individuals increased 20% after one week on a low FODMAP diet, which is contrary to the expected reduction in volume that might occur secondary to reduced gas production (Major et al., 2015a). In fact, fasted expired hydrogen was reduced in this group suggesting that the increased colonic volume was independent of changes in gas and it was speculated that diet-induced alterations in the microbiota were responsible. Further work is needed to confirm these findings in patients with IBS.

Given the effects of FODMAPs in the GI tract, it is plausible that dietary restriction of these carbohydrates might be effective in ameliorating IBS symptoms, particularly in patients with visceral hypersensitivity. Limiting luminal distension through reducing small intestinal water and colonic gas production would reduce sensory afferent input from the enteric system. Furthermore, the additive effect of these carbohydrates would suggest that collective restriction may improve symptoms more than restriction of one or two individual carbohydrates.

1.2.4 Structure and delivery of the low FODMAP diet

The low FODMAP diet is gaining widespread acceptance in primary, secondary and tertiary centres for the management of IBS. Data on clinical effectiveness of the low FODMAP diet is available only for dietitian-led advice and therefore it is unknown whether other methods of delivery (e.g. leaflet, advice from non-dietetic health professional) are as effective.

Due to the complexity of the low FODMAP diet, the structure and delivery of advice is important for effectiveness and to ensure nutritional adequacy is achieved. Following careful assessment of medical, symptom and diet history, explanation is provided regarding the underlying mechanisms for the diet's effectiveness. Specific individualised advice regarding low FODMAP fruit, vegetables, grains and dairy products is provided and is supported with complementary written information listing suitable and unsuitable foods. Advice regarding avoidance of added high FODMAP ingredients (e.g. inulin), food product label reading, eating out and maintaining a varied balanced diet is essential. Routine practice involves the restriction of FODMAPs for at least 4 weeks, which is followed by a systematic reintroduction phase if sufficient symptom response occurs. Each FODMAP carbohydrate is challenged in increasing doses to determine tolerance. Nutritional adequacy should be assessed throughout the entire process and addressed where necessary.

1.2.5 Clinical effectiveness of the low FODMAP diet

The following sections will review the evidence for the clinical effectiveness of the low FODMAP diet in relation to GI symptoms and HRQOL in IBS.

1.2.5.1 GI symptoms

The last decade has seen publication of numerous studies investigating the effect of a low FODMAP diet on IBS symptoms. Publication of two recent systematic reviews, albeit with

different conclusions, confirms the growing research interest in the area (Rao et al., 2015, Marsh et al., 2015). Many of the studies are limited due to their retrospective and/or uncontrolled observational nature, however some RCTs have been undertaken which report promising findings.

The first retrospective study of the low FODMAP diet ever published undertook a case review of 62 IBS patients a median of 10 months after initial dietary consultation. Patients were advised to restrict fructans and fructose and some patients also received advice regarding avoidance of other FODMAPs. In total, 77% of patients reported improvement in symptoms, with an impressive majority of 85% of adherent patients reporting benefit (Shepherd and Gibson, 2006). Other prospective, uncontrolled studies support these findings especially for overall symptoms, pain, bloating and diarrhoea (Wilder-Smith et al., 2013, de Roest et al., 2013).

Our group evaluated the effect of dietitian-led low FODMAP dietary advice compared with standard dietary advice based on national guidelines (NICE, 2015) in a non-RCT of IBS patients (Staudacher et al., 2011). More patients (76%) reported satisfaction with symptom response compared with those receiving standard advice (54%) after 2-6 months ($p<0.05$), although follow up only included those patients who returned to clinic. Retrospective, uncontrolled and non RCTs are limited by obvious shortcomings associated with this type of research, including a lack of control of external factors that impact on symptom experience (e.g. drugs, diet), bias associated with non-random allocation and the inability to distinguish an effect of treatment over placebo. Furthermore, most studies of this nature do not measure dietary intake or compliance.

Six RCTs have been undertaken investigating the effectiveness of a low FODMAP diet in adults with IBS (**Table 1.6**), of which three were unblinded. In these studies, patients randomised to low FODMAP dietary advice for 4-6 weeks reported improvements in a variety of GI symptoms based on various symptom scoring tools (Staudacher et al., 2012, Pedersen et al., 2014, Harvie et al., 2013). Dietary intake was assessed in two of the three studies which confirmed a reduction in FODMAP intake, and only one reported no adverse alteration in other dietary components (e.g. fibre) that might bias symptom outcomes (Staudacher et al., 2012). There are inevitable problems associated with unblinded intervention studies in IBS as preconceived expectations about a treatment may prime patients to sense and record symptom outcomes

Table 1.6 RCTs investigating the effectiveness of a low FODMAP diet in adults with IBS

Reference	Study design	Participants	Duration	Adherence	Symptom scoring	Findings
Unblinded						
(Pedersen et al., 2014)	Randomised unblinded controlled trial (dietary advice)	Rome III IBS LFD n=42 LGG n=41 habitual diet n=40	6 weeks	No	Web accessed IBS-SSS	8/42 (20%) low FODMAP withdrew due to difficulty of diet, 4/42 LGG withdrew ↑IBS-SSS total score reduction low FODMAP (75 pts) compared with control (32 pts; p<0.01) but not compared with probiotic (32 pts, p=0.2) ↓IBS-SSS score for all subscores low FODMAP vs baseline
(Harvie et al., 2013)	Randomised controlled trial (dietary advice)	Rome III IBS waiting list n=27 LFD n=23	3 months	FODMAP FFQ	IBS-SSS	↑reduction in IBS-SSS score low FODMAP (276 to 129 pts) compared with control (247 to 204 pts; p < 0.01) ↑ IBS-SSS Δ in episodes pain low FODMAP compared with control (p < 0.01)
(Staudacher et al., 2012)	Randomised controlled trial (dietary advice)	Rome III IBS with bloating or diarrhoea habitual diet n=22 LFD n=19	4 weeks	7-day food diary	AR question GI Symptom Rating Scale Bristol Stool Form Scale	3/22 (14%) low FODMAP withdrew ↑% patients reporting AR low FODMAP (68%) vs control (23%; p=0.005) ↑% patients reporting improvement in bloating, borborygmi, urgency, overall symptoms low FODMAP vs control (p<0.05) ↑% normal stool consistency low FODMAP vs control (24% vs 7%, p=0.02)
Blinded						
(Bohn et al., 2015)	Randomised blinded controlled trial (dietary advice)	Rome III IBS LFD n=38 standard diet n=37	4 weeks	4-day food diary	IBS-SSS Stool frequency and consistency Responder: ≥ 50pt reduction total IBS-SSS	4/38 (11%) low FODMAP diet withdrew, 3/37 (8%) standard diet withdrew ↓total IBS-SSS score compared with baseline for low FODMAP diet (p<0.01) and standard diet (p<0.01) ↓IBS-SSS subscores compared with baseline for pain frequency, severity of distension and life interference for both groups (p<0.01) 50% responders low FODMAP diet, 46% responders standard diet (p=0.72) ↓stool frequency low FODMAP diet vs baseline (1.9 vs 1.5/day, p<0.001)
(Piacentino et al., 2015) (Abstract only)	Double blind randomised controlled trial	Rome III IBS n=75 LFD-GF LFD or normal diet	4 weeks Follow up at 16 months	Not specified	AR question Bloating VAS Satisfactory relief VAS	↑improvement in intensity and frequency of abdominal bloating in LFD-GF and LFD vs habitual (p<0.001) No difference between groups for AR ↑Satisfactory relief in LFD vs LFD-GF using VAS (p=0.044)
(Halmos et al., 2014)	Randomised, single blind, controlled crossover trial (feeding study)	Rome III IBS n=27 Australian diet vs LFD	21 days	7-day food diary	100 mm VAS for 4 symptoms and overall Stool frequency Stool water content	↓overall GI symptoms low FODMAP diet (23 mm) vs typical Australian diet (45 mm; p<0.001). Similar outcomes for bloating, pain, stool dissatisfaction 70% low FODMAP >10 mm reduction in overall GI symptoms ↓Reduced stool frequency in IBS-D low FODMAP diet vs Australian diet

LFD, Low FODMAP diet; LGG, *L. rhamnosus* GG; FFQ, food frequency questionnaire; IBS-SSS, IBS Severity Scoring System; AR, adequate relief; LFD-GF, low FODMAP diet and gluten free

differently. This is a particular problem in IBS where outcomes are subjectively assessed and are highly sensitive to participant behaviour and where placebo effect is considerable (20-40%) (Elsenbruch and Enck, 2015).

Blinded dietary intervention studies are notoriously difficult to perform. In low FODMAP studies to date, two methods have been employed to attempt to blind patients to the dietary intervention. One single blind crossover feeding study provided all food and fluid to patients for two 21-day feeding periods. Overall symptoms, pain, bloating and flatulence were significantly lower in response to low FODMAP feeding compared with patients were fed a typical Australian intake. Improvement in overall GI symptoms, demonstrated by at least a 10 mm reduction on a visual analogue scale (VAS), was observed in 70% of patients, and symptoms were noted to improve in the first week of treatment (Halmos et al., 2014).

An advantage of feeding studies is the ability to carefully control dietary intake and compliance to the intervention, however controlled feeding does not mimic the real-life challenges associated with free living individuals having to sustain a restricted diet. Furthermore, crossover studies carry with them questions regarding the minimum wash out period required between interventions, and the effect on patient perception on allocated treatments. Another specific issue in this study was the increase in symptoms in the control group, which may have been due to an increase in FODMAP intake compared with habitual diet, leading to an artificially greater symptom difference between the groups (Halmos et al., 2014). Nevertheless, this was the first placebo-controlled low FODMAP intervention trial and an important contribution to this area of research.

The second blinded study (abstract only) compared the effect of a low FODMAP diet with and without gluten with controls following normal diet (n=75) (Piacentino et al., 2015). The implementation of the diet was unclear, however it was double blind which suggested it was a controlled feeding study. Global response measured using a VAS was higher in patients consuming a standard low FODMAP diet compared with those consuming a combined low FODMAP-gluten free diet (p=0.044). This is the first evidence that low FODMAP advice alone is superior to a more restrictive approach. Interestingly, more patients continued the low FODMAP diet at the 16-month follow up (72%) compared with the low FODMAP-gluten free group (52%) further suggesting gluten avoidance in addition to the low FODMAP diet did not offer additional benefit.

The third blinded study investigating the effect of the low FODMAP diet on symptoms in IBS was a recent large two-arm parallel design RCT conducted in Sweden (Bohn et al., 2015). Patients were randomised in a single blind fashion to either low FODMAP advice (n=38) or standard dietary advice (n=37) based on UK clinical guidelines (NICE, 2015) for 4 weeks. Symptom outcomes were measured using the IBS Severity Scoring System (IBS-SSS) and dietary compliance was evaluated by quantification of FODMAP intake based on 4-day food diaries. Clinical response, a 50-point reduction in IBS-SSS score, was demonstrated in both groups (50% low FODMAP vs 46% standard, p=0.72) and groups demonstrated comparable reduction in score compared with baseline. These results suggest that the low FODMAP diet may be no more effective than standard advice which contrasts with results of a large unrandomised controlled trial comparing the same two interventions (Staudacher et al., 2011).

The lack of an effect of the low FODMAP diet above standard advice in this RCT could be explained in a number of ways. Firstly, the RCT included all subtypes of IBS patients, and IBS-C comprised 25-35% of the entire cohort. Although published data are not available, patients with IBS-C may be less likely to respond to the low FODMAP diet, which in part might explain these findings. Secondly, this RCT was the first study to include IBS severity as an inclusion criterion, whereby patients required a score of at least 175 points (moderate severity) on the IBS-SSS. Therefore, this cohort may have been different to previous studies where baseline symptom severity was more variable. Thirdly, baseline FODMAP intake was lower than that reported in other work (Staudacher et al., 2012), although not all (Halmos et al., 2014), which decreased the absolute reduction in dietary FODMAP load that was possible. Clinical response to a low FODMAP diet is subject to the accuracy of the advice provided and the source of information used to formulate the low FODMAP diet was not specified in this study. Therefore the dietary advice may have been somewhat different to that implemented in previous studies. Finally, the standard advice provided in this and prior work was based on UK dietetic advice which involves avoidance of a number of foods (e.g. Brussels sprouts, sugar free gum), many of which are high in FODMAPs, which led to a significant reduction in the intake of fructose and a trend toward a reduced intake of GOS in the standard group in this study, which may have masked a true treatment effect in the low FODMAP group. A strategy that avoids this problem would be the use of 'sham' dietary advice devised to maintain FODMAP and nutrient intake whilst remaining a convincing exclusion diet (see Chapter 3). Finally, the results

of this RCT may in fact have reflected a true lack of response in this cohort, and replication of these findings in future RCTs is required to verify whether this is the case.

In summary, current evidence suggests up to 70% of patients with IBS report symptomatic benefit on a low FODMAP diet. Indeed, the first meta-analysis of low FODMAP RCTs reports effects on abdominal pain (OR 1.81), abdominal bloating (OR 1.75) and overall GI symptoms (OR 1.81) (Marsh et al., 2015). In line with these overall findings, national guidelines for the dietary management of IBS in the UK now advise consideration of a low FODMAP diet if basic diet and lifestyle measures have been unsuccessful in managing symptoms (NICE, 2015). However, until now, the impact of the low FODMAP diet on IBS symptoms has not been confirmed in a blinded placebo-controlled dietary advice RCT.

1.2.5.2 HRQOL

The effect of a low FODMAP diet on HRQOL in patients with IBS has been explored in a limited number of studies. Some studies demonstrate improvement in disease-specific HRQOL in patients with IBS after low FODMAP dietary advice (Ostgaard et al., 2012, Mazzawi et al., 2013) whilst others show no effect (Pedersen et al., 2014). All studies that have measured HRQOL have, however, been hindered by lack of blinding, have been uncontrolled in nature or implement broad dietary changes rather than low FODMAP dietary advice alone. One unblinded RCT has evaluated HRQOL in response to low FODMAP intervention. Both IBS-SSS scores and IBS-QOL improved compared with controls at 3 months, although whether medication and other lifestyle factors that might impact on HRQOL were controlled was not specified (Harvie et al., 2013). It is known that HRQOL can improve in response to placebo (Eickhoff, 2008), and therefore a placebo-controlled RCT is required to confirm whether low FODMAP dietary advice does indeed impact on HRQOL.

1.2.6 Impact of the low FODMAP diet on the GI microbiota

Despite the beneficial clinical effects of a low FODMAP diet in IBS, some potentially unfavourable consequences may result from this type of dietary manipulation. In particular, implementation of the low FODMAP diet results in a considerable reduction in intake of prebiotic fructans and GOS (Staudacher et al., 2012, Bohn et al., 2015). This represents a considerable reduction in total carbohydrate substrate available for colonic fermentation. In line with data from other studies that impose significant dietary restriction, this is likely to

have repercussions on the composition and functioning of the GI microbiota. Four studies have investigated whether this is the case in patients with IBS.

The first study, undertaken by our group, investigated the effect of a 4-week low FODMAP diet on stool microbiota in IBS patients with bloating or diarrhoea using fluorescence *in situ* hybridisation (FISH) (Staudacher et al., 2012). A reduction in total FODMAP intake of 50% led to a marked 6-fold reduction in the relative abundance of Bifidobacteria compared with controls who maintained FODMAP, macronutrient and fibre intake whilst following habitual diet. This microbiota alteration was inversely associated with baseline Bifidobacteria concentration, such that those with higher baseline concentration exhibited a greater reduction in abundance. This was a novel finding, although the reverse has previously been demonstrated with prebiotic supplementation (Whelan et al., 2005). There were no differences in total bacteria or other bacteria such as Lactobacillus or *F. prausnitzii*, or fermentation byproducts such as stool SCFA concentration or stool pH between groups.

The second study investigated the effect of a 3-week low FODMAP diet on the gut microbiota using quantitative polymerase chain reaction (qPCR) and also demonstrated a reduction in absolute Bifidobacteria concentration (Halmos et al., 2014). This was accompanied by substantial reduction in total bacterial load of 47% compared with habitual diet, as well as reduction in absolute abundance of *F. prausnitzii* and Clostridium Cluster IV. Diversity of Clostridium Cluster XIV was greater after low FODMAP intervention compared with habitual diet, which was postulated to be due to species adaptation to altered substrate availability. This was a crossover study, and therefore the potential of carryover effects cannot be ruled out. Furthermore, microbiota data from the patients with IBS was pooled with a group of healthy controls (n=6), potentially concealing differences between the groups in terms of response to the dietary intervention.

Two studies have recently investigated the effect of a low FODMAP diet on the GI microbiota in children. One uncontrolled study found no effect of a 1-week low FODMAP diet on overall diversity or abundance of specific bacterial groups based on 454 pyrosequencing (Chumpitazi et al., 2014). Another specifically assessed whether symptomatic response to two days of a low FODMAP diet, based on pain frequency, was predicted by microbiota at baseline or by diet-induced changes to the microbiota (Chumpitazi et al., 2015). This was a crossover feeding study, and symptom response occurred in only 24% of patients. However, increased baseline

abundance of taxa such as Bacteroides, *Ruminococcaceae* and *F. prausnizii*, were associated with response, suggesting patients with a higher abundance of saccharolytic microbiota may benefit the most from a reduction in dietary fermentable substrates. No such association has been demonstrated in adult patients (Halmos et al., 2014), and more data are required in longer duration parallel-arm trials.

Until now there has only been one parallel group RCT in adults investigating the effect of a low FODMAP diet on a limited number of bacterial groups. Clearly, there is still much to understand regarding the impact of this dietary approach on the GI microbiota. Importantly, it is not yet known whether the mucosal compartment is affected, or if there is a critical timepoint at which microbiota alterations might have functional consequences and whether these changes lead to short or long term health consequences. Further investigation into the relevance of microbiota composition in predicting symptom response to the low FODMAP diet is needed, and will require an agreed definition of 'response'. Reintroduction of FODMAPs to tolerance may attenuate some of the diet-induced microbiota alterations, but this has never been explored.

1.2.7 Impact of the low FODMAP diet on nutrient intake

The clinical effectiveness of a dietary intervention must always be weighed against the impact it has on the ability of patients to maintain appropriate nutrient intake in the long term. The ramifications of an exclusion diet are, to a certain extent, dependent on the baseline diet of the patient group in question. A number of cross sectional studies have examined habitual dietary intake of individuals with IBS and report that patients meet nutrient requirements, including for calcium and folate (Bohn et al., 2013, Williams et al., 2011), and do not differ from healthy controls (Saito et al., 2005). With relation to intake of individual foods, there is some data to suggest that milk intake is lower in IBS versus healthy controls (Hayes et al., 2014) although this is not a consistent finding (Saito et al., 2005). Therefore, from the evidence to date, it appears nutrient intake of patients with IBS meets nutrient recommendations and is not significantly different to healthy controls.

Measurement of dietary intake in low FODMAP intervention trials is only sometimes undertaken to confirm dietary adherence to the intervention, and to ensure minimal modification of other important components such as fibre that might impact on symptoms. In relation to macronutrients, two dietary advice studies report that low FODMAP dietary advice

reduces total carbohydrate intake compared with habitual diet to 150-200 g/d (Staudacher et al., 2012, Bohn et al., 2015), which is reflective of carbohydrate intake in healthy individuals in the UK (Public Health England and Food Standards Agency, 2014). One study has reported a reduction in energy, protein and fat intake in patients following low FODMAP advice (Bohn et al., 2015), which may be of concern particularly if the diet is followed in the longer term.

One RCT to date has examined micronutrient intake in patients following low FODMAP dietary advice (n=16) (Staudacher et al., 2012). Iron intake according to 7-day diet records was not different to controls following habitual diet, suggesting that iron-fortified wheat breakfast cereals and other iron-rich foods (e.g. pulses, nuts) were adequately substituted. However, a lower calcium intake was reported compared with controls (600 vs 730 mg/d, p=0.016), and although food group consumption was not reported, it is likely that this was due to restriction of high lactose foods (e.g. milk) with insufficient replacement of high calcium alternatives. Measurement error due to lack of low lactose food composition data in dietary analysis software may also be a contributing factor, as this could lead to underestimation of micronutrient intake. Finally, mean intake data may be skewed by low or high intakes, which would mask true average dietary intakes of a cohort, whereas data presented as the proportion of patients meeting nutrient requirements may be more meaningful. Nevertheless, further larger studies are required to confirm whether the intake of calcium and/or other micronutrients is compromised when patients with IBS follow a low FODMAP diet.

1.2.8 Conclusion

The low FODMAP diet is an emerging therapy that leads to symptom improvement in nearly three quarters of patients with IBS. Importantly, robust evidence is available for the mechanisms underlying its effectiveness. A blinded dietary advice RCT is required to measure its effect over placebo in a clinically relevant way, and its impact on HRQOL and nutrient intake requires further exploration. Furthermore, whether a simultaneous intervention could prevent the low FODMAP diet-induced GI microbiota alterations has never been investigated.

1.3 Dietary approaches to modifying the microbiota

1.3.1 Prebiotics

A prebiotic is 'a selectively fermented ingredient that results in specific changes in the composition and/or activity of the GI microbiota, thus conferring benefit(s) upon host health' (Gibson et al., 2010). Beneficial effects of prebiotics include improved calcium absorption and bone health, enhanced lipid metabolism (Roberfroid et al., 2010), reduced risk of overweight (Perez-Cornago et al., 2015) and reduced risk of the development of colon cancer in animal models (Roberfroid et al., 2010). Furthermore, prebiotic supplementation, in contrast to the low FODMAP diet where some prebiotic substrates are restricted, may be useful in IBS where dysbiosis has a potential aetiologic role. The compounds identified as having the most evidence for prebiotic effects are the inulin-type fructans (FOS, inulin and oligofructose) and GOS, many of which are widely distributed throughout the diet predominantly in grains, vegetables and pulses. Food and commercial sources of prebiotics are described in **Table 1.7**.

Table 1.7 The composition, source and structure of prebiotic carbohydrates

Prebiotic	Composition	Commercial source	Food source	DP
Inulin	β(2-1) fructans	Chicory root	Wheat, garlic, onion, leek, bread	11-65
Oligofructose	β(2-1) fructans	Chicory root		≤ 10
FOS	β(2-1) fructans	Sucrose		2-10
		Chicory inulin		3-5
GOS	Oligo-galactose (85%), glucose, lactose	Lactose	Beans and pulses	2-5
Soya-oligosaccharides	Raffinose and stachyose	Soya bean whey	Soya beans	3-4
Xylo-oligsaccharides	β(1-4) linked xylose	Xylan	-	2-4
Pyrodextrins	Glucose-containing oligosaccharides	Potato or maize starch	-	Various
Isomalto-oligosaccharides	α(1-4) glucose, branched α(1-6) glucose	Maltose	-	2-8
Lactulose	Fructose and galactose	Lactose	-	2

DP, degree of polymerisation. Adapted (Macfarlane et al., 2006)

Total daily dietary intake of inulin and oligofructose in UK and Europe in healthy individuals is 4 g/d and 10 g/d, respectively (Dunn et al., 2011, van Loo, 1995). Due to their indigestibility in

the human small intestine, prebiotics become available for colonic bacterial fermentation. Prebiotic carbohydrates with a smaller DP produce fermentation byproducts (SCFA, gas) at a higher rate than those with a larger DP (Hernot et al., 2009). The bifidogenic effect (the extent to which growth of Bifidobacteria is stimulated) of inulin and oligofructose is inversely associated with baseline Bifidobacteria concentration *in vivo* (Whelan et al., 2005). The magnitude of response appears to be highly variable and reverses within a week of cessation of supplementation (Davis et al., 2011), but prebiotic supplementation may be a therapeutic option for IBS, where dysbiosis, including reduced stool and mucosal Bifidobacteria concentration is a common feature.

Prebiotic supplementation studies usually supplement background dietary prebiotic intake with an additional 5-20 g/d, essentially at least doubling prebiotic intake in most individuals. There are at least four RCTs investigating supplementation of prebiotics in adults with IBS or FBD. Two studies have found no effect of prebiotic supplementation of 6 g/d oligofructose for 2 weeks (Hunter et al., 1999) or 20 g/d FOS for 12 weeks (Olesen and Gudmand-Hoyer, 2000) in IBS compared with placebo. In fact, symptoms were worse compared with placebo at four weeks in the latter study. In the third and largest study, 106 patients with new-onset, minor, functional bowel symptoms were randomised to receive 5 g/d oligofructose or placebo for six weeks (Paineau et al., 2008). Intensity and frequency of symptoms was reduced compared with placebo, however a major limitation of this study was the absence of an intention-to-treat analysis, which is significant as approximately half of the recruited sample were poorly compliant and excluded from the analysis.

The most recent RCT of prebiotics in IBS recruited 60 patients to assess the effect of β-GOS on symptoms. It was the only study to assess the impact on the microbiota, confirming a bifidogenic effect in patients receiving either 3.5 g/d or 7 g/d for four weeks. The low dose group demonstrated improvement in a number of symptoms compared with baseline and placebo, and the high dose group also reported improvement in global score, although there was also a significant increase in bloating. This study is also limited due to the absence of an intention-to-treat analysis, and therefore did not account for the 16/60 patients who withdrew from the trial (Silk et al., 2009).

Overall there is minimal evidence for the effectiveness of prebiotic supplementation for the management of IBS symptoms. Indeed none of the above studies were included in a recent

systematic review and meta-analysis of prebiotics in IBS (Ford et al., 2014b), and a withdrawal rate of 25-50% in the most recent studies might lead one to question the patient acceptability of prebiotic therapy in IBS. The dose at which luminal distension from increased fermentative gas production worsens symptoms needs evaluation. Furthermore, work is required to clarify whether there is a role for prebiotics in a subset of patients with IBS, and in particular whether there is a role for prebiotic carbohydrates that modulate the microbiota without substantially increasing colonic gas production.

1.3.2 Probiotics

Probiotics present another means of modulating the GI microbiota in IBS in order to improve GI symptoms. The following sections will describe the factors that are important in the selection of a probiotic, the mechanisms by which they might have an effect on GI function, and the evidence for the clinical effectiveness of probiotics in IBS.

1.3.2.1 Definition

Élie Metchnikoff first attributed health benefits to Lactobacilli in yoghurt in 1907. These benefits were thought to be mediated through alteration of the colonic microbiota. In 1965, the term probiotic was devised, meaning 'for life' in Greek. Subsequent refining of the term has led to a precise definition that encompasses viability and a benefit to health. The current Food and Agriculture Organisation of the United Nations (FAO) and World Health Organisation (WHO) probiotic definition is 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' (Hill et al., 2014).

Probiotic benefit is proposed to occur as a result of adhesion, colonisation and action at the level of the colonic epithelium, although benefit can be conferred in the absence of adhesion and proliferation (Hill et al., 2014). Formulation of a probiotic product involves isolation from the original source (e.g. food, human breast milk, faeces), enumeration and safety testing (Fontana et al., 2013). The most common probiotic organisms are Bifidobacteria, Lactobacilli or *Saccharomyces boulardii* and these are widely available over-the-counter in capsule, liquid or powdered form, or as addition to food, such as in yoghurt or fermented milk drink.

1.3.2.2 Probiotics in health and disease

Probiotic therapy has been the subject of intense preclinical and clinical research in health and a variety of disease states. The role of probiotics as metabolic modulators in obesity, type 2

diabetes and the metabolic syndrome, and in the treatment and prevention of non-GI conditions such as acute respiratory infection, allergy, autoimmune disease and psychiatric disease is a major research focus. Considerable work has concentrated on the potential role of probiotics in disorders of the GI tract and currently there is evidence for their effectiveness in lactose malabsorption, antibiotic-associated diarrhoea (Scott et al., 2015), pouchitis and induction and maintenance of remission in ulcerative colitis (Shen, 2014). Although there has been considerable research on probiotics in IBS, a surprisingly large proportion of the published literature comprises reviews or commentaries rather than robust clinical trials of individual products.

1.3.2.3 Viability of probiotics

A key requirement of probiotics, according to the definition, is that the organisms are 'live' or viable. Non-viable bacteria that might have positive effects *in vivo* mediated through bacterial DNA or cell wall components (Rijkers et al., 2010) do not therefore fit within the current probiotic definition. A minimum dose of organisms is also not required under the current definition, although $1x10^9$ colony forming units per serving is required in some countries for product labelling purposes (Hill et al., 2014).

Probiotic viability can be assessed *in vitro* or *in vivo*. *In vitro* evaluation is performed by subjecting the organism(s) to adverse low pH conditions representative of the stomach (pH 1.5-3.0) and bile salts that have antimicrobial effects (Fontana et al., 2013). Alternatively, enhanced stool recovery of the probiotic organism(s) after a period of oral supplementation is a measure of viability *in vivo*. Organism survival *in vivo* as a proportion of overall dose is relatively low (15-45%) (Del Piano et al., 2006).

Probiotic survival through the GI tract is influenced by a number of host and product-specific factors. The major host-specific factors include genotype, age, diet and baseline microbiota composition. The contribution of each host-specific factor to determining probiotic viability is difficult to ascertain as they are inextricably linked. For example, diet shapes the microbiota, and ageing influences dietary intake and the microbiota. A number of probiotic-specific factors are important in influencing viability of the microorganisms in the product. These include the delivery matrix, the dose and the organism itself. For example, one problem with high dose probiotics is their susceptibility to suboptimal physical characteristics (e.g. dissolution)

compared with lower doses, which could impact on availability in the colon, and this requires consideration throughout product formulation and quality control (Whorwell et al., 2006).

There are a number of probiotic products available in the UK with demonstrated viability (**Table 1.8**). VSL #3 is a high dose probiotic containing eight bacterial strains, including Bifidobacteria and Lactobacilli. It has been the most researched of all the UK available products, and its viability has been demonstrated. For example, supplementation with 900 billion bacteria/day led to increased stool concentration of the strains *B. infantis* Y1 and *B. breve* Y8 and total Bifidobacteria in healthy individuals after three days in one small study (Brigidi et al., 2003). Furthermore, four weeks of supplementation at the same dose in patients with IBS has been shown to increase mucosal abundance of Bifidobacteria and Lactobacilli (Ng et al., 2013). Conversely, two studies have demonstrated a lack of effect of on stool microbiota after high dose supplementation in patients with IBS-C (Kim et al., 2015) and IBS-D (Michail and Kenche, 2011). These conflicting findings may be attributed to differing methodologies used to quantify the microbiota, inherent differences between IBS subtypes studied, or confounding factors such as baseline diet.

Table 1.8 Probiotic products with demonstrated viability available in the United Kingdom

Product	Total dose	Composition	Reference for viability
Alflorex	1 billion/capsule	*B. infantis* 35624	(Charbonneau et al., 2013)
Actimel	10 billion/100g	*L. casei* DN 114 001	(Rochet et al., 2006)
Activia	4 billion/125g	*B. actiregularis*	(Rochet et al., 2008)
VSL#3	450 billion/sachet 112.5 billion/capsule	*S. thermophilus* *B. breve* *B. longum* *B. infantis* *L. acidophilus* *L.plantarum* *L. paracasei.* *L. delbrueckii subsp. bulgaricus*	(Brigidi et al., 2000, Brigidi et al., 2001, Brigidi et al., 2003)
Yakult	6.5 billion/65ml	*L. casei* Shirota	(Fujimoto et al., 2008)

1.3.2.4 Adhesion and persistence

Adhesion of a probiotic organism to the colonic mucosa promotes longer lasting residence in the colon and may also be essential for specific probiotic effects including pathogen exclusion and immunomodulation. Adhesion is a complex process and the cell components involved

have not been well examined. The duration of probiotic persistence in the colon on cessation of supplementation is also unclear. Stool recovery of the probiotic strains B. breve and B. infantis has been demonstrated up to six days following cessation of VSL#3 supplementation using culture methods (Brigidi et al., 2003). Furthermore, one recent study in IBS assessed stool microbiota composition throughout and following eight weeks of supplementation with *B. infantis* 35624 (Charbonneau et al., 2013). Peak concentration of the strain occurred at eight weeks and returned to baseline two weeks after discontinuation of the probiotic, with no differences between healthy individual and patients with IBS (Charbonneau et al., 2013). Therefore, overall it can be assumed that the duration of persistence is not influenced by the presence of IBS but may vary between probiotic products.

1.3.2.5 Mechanisms of probiotic action in IBS

Probiotics have various proposed modes of action in IBS. One model has suggested probiotics act at three levels: the GI lumen, the colonic mucosa, and the nervous system (**Figure 1.6**) (Rijkers et al., 2010). The following section describes the various mechanisms of action by which probiotics might target IBS symptoms at these three levels.

1.3.2.5.1 The lumen: Microbiota and microbiota byproducts

Probiotics might improve IBS symptoms via direct augmentation or alteration of the commensal microbiota, which is known to be altered in a subset of patients with IBS (Section 1.1.7.4). In effect, probiotic bacteria might replace a 'missing part' of the commensal microbiota, either in the small and/or large intestine, or stimulate a component of the existing commensal population (Scott et al., 2015). In doing so, functionality of the microbiota might be restored, leading to improvement of symptoms. This could occur through a variety of local pathways, such as competitive exclusion of other bacteria, the production of antibacterial bacteriocins or alteration in the fermentation capacity of the microbiota. The effect of probiotics probably extends further than modification of the commensal microbiota, as functional alterations have been identified using new genomic and metabolomic techniques in the absence of changes to microbiota composition (Scott et al., 2015).

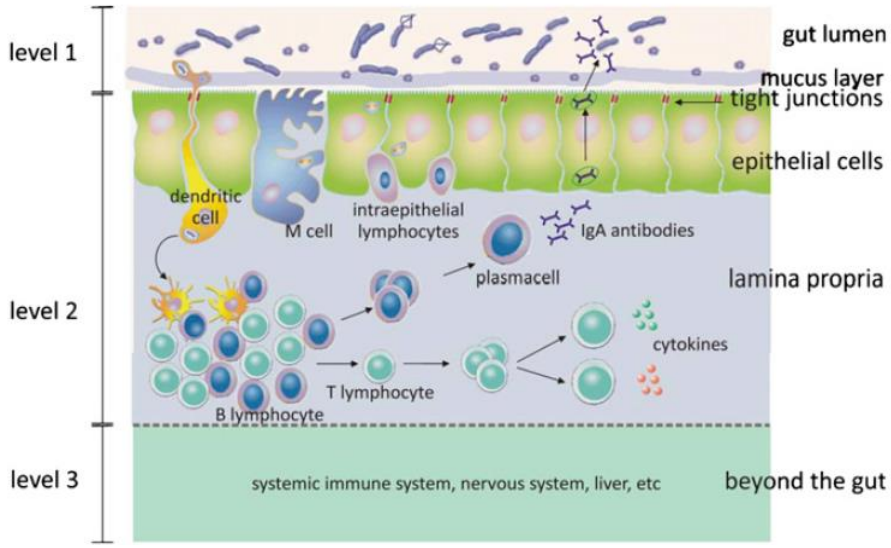

Figure 1.6 Levels of action of probiotics: GI lumen, mucosa, nervous system (Rijkers et al., 2010)

1.3.2.5.2 The lumen: GI transit

Probiotics may exert an effect on GI transit, and thereby influence symptoms in IBS. For example, *L. paracasei* probiotic has been shown to attenuate hypercontractility induced by infection in a mouse model, although the same was not demonstrated for four other Lactobacillus and Bifidobacteria strains (Verdu et al., 2004). A recent meta-analysis of human studies reported an overall effect of probiotics on decreasing transit time (Miller and Ouwehand, 2013), although only two studies were in IBS, and both of these evaluated IBS-C patients. Furthermore, VSL#3 supplementation has been shown to increase transit time in IBS (Kim et al., 2005) but in another study it had no effect compared with placebo (Kim et al., 2003). Therefore, the effect of probiotics on transit is variable, and may differ between IBS subtypes and probiotic products. Modulation of the microbiota and its metabolic output by probiotics (e.g. fermentation byproducts) may be important in mediating the effect on transit (Barbara et al., 2005, Ringel-Kulka et al., 2015), however this is difficult to demonstrate in isolation, as the microbiota, fermentation byproducts and GI transit are interdependent (Kashyap et al., 2013, Barbara et al., 2005, Ringel-Kulka et al., 2015).

1.3.2.5.3 The mucosa: Intestinal permeability and inflammation

It has been proposed that probiotics may improve IBS symptoms through attenuation of impaired intestinal permeability and the accompanying low grade immune activation. *In vitro* and animal studies have demonstrated probiotics influence tight junction protein expression

and increase the number of tight junction protein structures. For example, VSL#3 treatment for seven days leads to preservation of the expression of the tight junction proteins occludin and zonula occludins-1 (ZO-1) in colonic tissue of IBS rat models compared with placebo, which is associated with preservation of intestinal permeability (Dai et al., 2012). Most evidence of this nature is from animal infection models which may not be representative of epithelial dysfunction in non PI-IBS.

Alteration in intestinal permeability in response to probiotic supplementation has been investigated in a small number of human studies. No difference in colonic intestinal permeability was found after four weeks of supplementation with a multispecies probiotic in one study of IBS-D patients. Small intestinal permeability was reduced compared with controls at four weeks, however, and this was accompanied by an improvement in global GI symptoms (Zeng et al., 2008). One further study investigated the effect of probiotics on inflammatory profile in IBS. Patients were randomised either to *L. salivarius* UCC4331 or *B. infantis* 35624 for eight weeks, and viability was confirmed by stool recovery (O'Mahony et al., 2005). Patients with IBS, who presented with a lower IL-10:IL-12 ratio at baseline, demonstrated changes to a level comparable with healthy controls at eight weeks. This was associated with an improvement in clinical symptoms, but both outcomes were only evident after *B. infantis* 35624 and not with the Lactobacillus probiotic. These results suggest probiotics might improve symptoms through modulating inflammatory profile, and the effect appears to be product-specific. More work is required to investigate the effect of probiotics on inflammation in IBS, and whether altered permeability is indeed an important mediator of these changes.

1.3.2.5.4 The nervous system: Visceral hypersensitivity

A number of animal studies suggest probiotics may reduce visceral hypersensitivity. For example, one study demonstrated that administration of *B. lactis* CNCM I-2494 dose-dependently inhibited stress-induced visceral sensitivity in rats compared with saline (Agostini et al., 2012). Likewise, another study reported that seven days of VSL#3 supplementation led to lower abdominal reflex scores over a range of balloon distension pressures compared with placebo (Dai et al., 2012). In both studies, improved visceral hypersensitivity was associated with preservation of intestinal permeability.

Human studies investigating the effect of probiotics on visceral hypersensitivity are limited. One 6-week RCT assessed the effect of a multispecies probiotic in patients with confirmed

visceral hypersensitivity based on rectal barostat measurements (Ludidi et al., 2014). The bacterial strains were chosen based on evidence supporting their impact on epithelial integrity and modulation of inflammatory markers, however rectal visceral hypersensitivity was not different between probiotic and placebo groups at six weeks. There was also no change in GI symptoms throughout progressive barostat pressures for either group. Conversely, a recent small study investigating VSL#3 demonstrated increased mean pressure thresholds for pain sensation in patients receiving VSL#3 compared with baseline, reflecting improvements in visceral hypersensitivity (Wong et al., 2015). There was no change for any other clinical parameters (e.g. first sensation of distension or urgency, pain tolerance) and mean scores compared with placebo were not reported. The positive effect of probiotics on visceral hypersensitivity from animal work has not robustly been replicated in humans, and more work is required to evaluate its effect in humans, and to determine whether improvements in visceral hypersensitivity, if any, are associated with reduced GI symptoms.

1.3.2.5.5 The nervous system: The brain

There has been recent growing interest in the role of probiotics on brain function. This may have implications in IBS as 1) there is impairment in the microbiome-brain-gut axis and 2) anxiety and depression are prevalent in IBS and may contribute to the pathogenesis of the condition.

A number of animal models elegantly demonstrate the effect of probiotics on the brain, and specifically on behaviour and stress response. For example, anxiety and depressive behaviours are attenuated in stress-induced mice fed probiotic versus controls (Bravo et al., 2011, Desbonnet et al., 2010). There is also some limited evidence of probiotic influencing anxiety behaviours in humans. For example, administration of *L. helveticus* R0052 and *B. longum* R0175 for 30 days was associated with a reduction in overall hospital anxiety and depression scale score, and other measures of stress and anxiety in healthy individuals compared with placebo (Messaoudi et al., 2011).

The first and only human study that has directly assessed brain function in response to probiotics was conducted in healthy female individuals (n=33) (Tillisch et al., 2013). Participants were allocated a milk drink containing Bifidobacteria and Lactobacillus species, dairy milk placebo or no intervention for four weeks. Intriguingly, lower activity was demonstrated in a number of brain regions in a negative attention task in the probiotic group

compared with controls. This included the periaqueductal gray, which is important in pain modulation (Behbehani, 1995). Whether this effect is evident in IBS and if IBS symptoms can be modulated via central mechanisms through probiotics has not been investigated.

1.3.2.6 Benefits and limitations of animal studies

Animal models are useful in reproducing some aspects of IBS for researching disease pathogenesis, underlying mechanisms and potential therapies. A number of models exist, including, 'restraint stress' which involves partial control of the animal's movements by taping upper limbs to its body. Some studies use a neonatal maternal separation model, based on the notion that exposure to stress experience early in life leads to visceral hypersensitivity. Other models include the post-inflammatory technique which involves generating GI inflammation by induction of colitis, or the use of antibiotic therapy to induce microbiota perturbations.

Much of the evidence for the mechanisms underlying probiotic effects in IBS stems from animal models and has not yet been extrapolated to humans in clinical trials. The complexity of the microbiota, dietary factors, stress response and coping mechanisms in humans are obviously distinct from animal models and they probably contribute to the disparity in animal versus human data in this area. Much further research is required for a complete understanding of the mechanisms of action of probiotics in patients with IBS.

1.3.2.7 Clinical effectiveness of probiotics in IBS

The abundance of studies investigating the mechanisms of action of probiotics, particularly in animals, is matched by a multitude of trials investigating their clinical effectiveness in patients with IBS. Indeed, eight systematic reviews and meta-analyses have been published in the last seven years. Systematic reviews and meta-analyses published in the last 5 years are detailed in **Table 1.9**. One recent meta-analysis is not included as it provided an analysis and summary results of grouped studies rather than pooling all findings (Didari et al., 2015).

Table 1.9 Systematic reviews and meta-analyses of probiotics in adults with IBS

Reference	Type	Patients	Number of studies	Outcome	Finding
(Ford et al., 2014b)	SR and MA	2575	23 RCTs	Dichotomous data: Persistence of symptoms after therapy	Benefit over placebo 56% probiotic vs 73% placebo RR 0.79 (0.70-0.89)
		2001	24 RCTs	Continuous data: Global symptom score or abdominal pain Flatulence Bloating Urgency	 Benefit over placebo SMD = -0.25 (-0.36,-0.14) Benefit over placebo SMD = -0.23 (-0.38, -0.07) No benefit over placebo SMD = -0.15 (-0.27, -0.03) No benefit over placebo SMD = -0.10 (-0.30, 0.10)
(Hungin et al., 2013)	SR	1313	11 RCTs	Primary outcome: Global symptoms Primary outcome: Abdominal pain Primary outcome: Bloating Primary outcome: Flatulence Primary outcome: Transit/stool frequency	5 of 9 studies found benefit over placebo 2 of 4 studies found benefit over placebo in IBS-D 4 of 6 studies found benefit over placebo 1 of 3 studies found benefit over placebo 0 of 3 studies found benefit over placebo 0 of 2 studies found benefit over placebo
(McKenzie et al., 2012)	SR	427	4 RCTs	Global symptom severity Flatulence	1 of 2 studies found benefit of *B. Lactis* DN 173010 over placebo 1 of 1 study found benefit of VSL#3 over placebo 1 of 2 studies found benefit of VSL#3 over placebo

SR, systematic review; MA, meta-analysis; RR, relative risk; SMD, standardised mean difference

The most recent rigorous systematic review of probiotic RCTs in IBS reported a marginal benefit for probiotic therapy in IBS compared with placebo. For the global dichotomous outcome analysis, the NNT for all probiotics was seven (Ford et al., 2014b), which is similar to the treatment benefit attributed to soluble fibre supplementation (Moayyedi et al., 2014). This review was also the first to sub-analyse the effect of individual products on IBS symptoms, reporting benefit for *L. plantarum* DSM 9843, *Escherichia* and *S. faecium* but not Bifidobacteria-containing products, although there was only a small number trials available for subgroup analyses. Other reviews cite evidence for probiotics improving overall symptoms and abdominal pain and bloating in IBS, a lack of evidence for flatulence (Hungin et al., 2013) and weak evidence of effectiveness for specific products in defined patient subgroups i.e. *B. lactis* DN 173010 in IBS-C patients, VSL#3 in patients with IBS and bloating (McKenzie et al., 2012).

The integration of data via systematic review and meta-analysis to estimate overall treatment effect is vital for the development of clinical guidelines, however debate exists as to whether meta-analyses are appropriate for probiotics in IBS (Whelan, 2014). Pooling data from studies that investigate a variety of probiotic organisms may obscure effects of certain strains or species. In fact, very few studies overlap with regards to probiotic composition, with the largest recent review including 35 RCTs that examined a total of 31 probiotic preparations (Ford et al., 2014b). Furthermore, significant heterogeneity exists between studies in relation to probiotic form, carrier product, duration of treatment and patient characteristics (e.g. IBS subtype, referral source). Control of concomitant IBS treatment and dietary intake, and measurement or reporting of adherence can vary widely (Ford et al., 2014b, Didari et al., 2015). Finally, and critically, studies can differ markedly in 'responder' definition. For example, many trials define response as symptom relief at a minimum of 50% of timepoints, whereas others measure response based on the IBS-SSS, and others use non validated scales.

Since the most recent meta-analysis described here, publication of probiotic RCTs in IBS continues at a rapid rate. At least 11 have been published in the last year, and approximately half of these showed a benefit for probiotic over placebo in IBS. There is fairly compelling data for a range of mechanisms in which probiotics impact on GI function via the microbiota, mostly from animal models, and this is accompanied by only moderate evidence for clinical effectiveness in IBS from human trials. RCTs investigating individual probiotic products in defined patient groups are needed to clarify their impact on specific GI symptoms as well as the optimum treatment regime to achieve benefit.

1.3.2.7.1 VSL #3 in IBS

VSL#3 is a widely used multispecies probiotic containing Bifidobacteria (*B. breve, B. longum, B. infantis*) and Lactobacillus species (*L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii* subsp*. bulgaricus*) and *S. thermophilus*. A total of six studies have examined the effect of VSL#3 supplementation in IBS, including one in functional diarrhoea (**Table 1.10**). The first two studies were uncontrolled in nature and reported 'clinical improvement' in a majority of patients following a 10-day supplementation period (Brigidi et al., 2001) and reduced stool frequency in patients with IBS-D (Bazzocchi et al., 2002).

Four VSL#3 supplementation RCTs have been conducted in IBS. Greater reduction in bloating (Kim et al., 2003) and lower flatulence scores (Kim et al., 2005) were reported after 4-8 weeks of varying doses of VSL#3 supplementation compared with placebo. One study demonstrated reduction in pain duration and distension severity compared with baseline in males, but no difference to placebo (Wong et al., 2015). Most RCTs evaluating VSL#3 in IBS have not measured stool outcomes and therefore this requires investigation in future studies.

Three of the six VSL#3 supplementation trials reported improvement in at least one IBS symptom in the patients studied. However, this evidence is less convincing when the study design flaws are considered, such as continued inclusion of participants requiring antibiotics (Kim et al., 2005, Kim et al., 2003), multiple reported incidences of medication intake in one study that may have impacted on symptoms (Kim et al., 2003), and a protocol alteration mid-study that reduced the intervention duration by half (Kim et al., 2005). Compliance was also infrequently reported. Finally, IBS symptoms were a primary endpoint in only one trial (Kim et al., 2005), and therefore the remaining studies may have been underpowered to detect symptom response.

Therefore, there appears to be some limited evidence that VSL#3 improves symptoms of bloating, flatulence and abdominal pain in IBS. However, the effect is not consistent across trials, and indeed no benefit was found for VSL#3 over placebo in a recent systematic review and meta-analysis (Ford et al., 2014b). Larger, more robust studies are required in homogenous IBS cohorts that use standardised outcome measures and evaluate compliance to confirm the place of VSL#3 in the management of IBS symptoms.

Table 1.10 Studies of VSL#3 supplementation in IBS

Reference	Study design	Participants	Dose/d	Duration	Primary outcome	Other outcomes	Finding
(Wong et al., 2015)	RCT	n=42 IBS (n=32 male) secondary care and community	500 billion bacteria	6 weeks	Not stated	Multiple symptoms (IBS-SSS)	↓IBS-SSS total score at follow up vs baseline probiotic 193 to 132 in males ($p<0.05$) ↓IBS-SSS days of pain and distension severity vs baseline in males ($p<0.05$)
(Michail and Kenche, 2011)	RCT	n=24 IBS-D source not stated	450 billion bacteria	8 weeks	Not stated	Multiple symptoms (GSRS) QOL (instrument not stated)	No differences
(Kim et al., 2005)	RCT	n=48 IBS Secondary care and community	900 billion bacteria in yoghurt	4/8 weeks	Weekly Satisfactory relief of bloating (50% of weeks)	Bloating, flatulence, pain, urgency (100 mm VAS)	Satisfactory relief bloating responders 46% probiotic vs 33% placebo ($p=0.27$) Flatulence placebo 39.5 mm vs probiotic 29.7 mm ($p=0.01$)
(Kim et al., 2003)	RCT	n=25 IBS-D secondary care	225 billion bacteria	8 weeks	Transit time	Weekly satisfactory relief (4 of 8 weeks = responder) Bowel function (consistency, frequency, ease of passage) Pain, bloating, flatulence, urgency (100 mm VAS)	Satisfactory relief 33% probiotic 38% placebo ($p=1.00$) Mean change in bloating probiotic -14 mm vs placebo -2 mm ($p=0.05$)
(Bazzocchi et al., 2002)	Uncontrolled	n=42 IBS-D source not stated	900 billion bacteria	20 days	Not stated	n/a	Reduction in mean stool frequency 7/d to 2/d ($p<0.002$)
(Brigidi et al., 2001)	Uncontrolled	n=10 IBS or FD source not stated	900 billion bacteria	10 days	Not stated	Urgency, abdominal discomfort, stool frequency and consistency	Clinical improvement in 9 of 10 but data not reported

IBS-SSS, IBS Severity Scoring System; GSRS, Gastrointestinal Symptom Rating Scale; QOL, quality of life; VAS, visual analogue scale

1.3.2.8 Probiotic safety in IBS

Probiotics generally have a sound overall safety profile. Some rare serious events (e.g. sepsis, metabolic complications) have been described in clinical trials in critically ill patients, and other trials report mild cases of abdominal pain, nausea, loose stools and flatulence (Doron and Snydman, 2015). A recent meta-analysis of probiotics in IBS reported an increased risk of adverse events compared with placebo (RR 1.21). The nature of the events was not detailed (Ford et al., 2014b), but it is plausible many were GI in nature. Clinical trials investigating new probiotic products must routinely collect safety data to confirm their safety profile for a variety of populations (Doron and Snydman, 2015).

1.3.2.9 Guidelines for use of probiotics in IBS

Clinical guidelines for the use of probiotics in IBS vary worldwide. The World Gastroenterology Organisation endorses probiotics as a primary intervention along with dietary and lifestyle considerations (World Health Organisation, 2009) whilst they do not feature in the Australian (Digestive Health Foundation, 2006) or US recommendations (Ford et al., 2014a). Meanwhile, a number of recommendations exist in the UK. Secondary care guidelines advise probiotics as second line therapy for bloating after diet and medications have been tested (Spiller et al., 2007), and dietetic guidelines advise their use as second line therapy (McKenzie et al., 2012). Specific guidelines for primary care advise probiotics as first line therapy alongside dietary and lifestyle advice for a period of 4 weeks at the dose recommended by the manufacturer (NICE 2015).

1.3.2.10 Patient acceptability and current clinical practice

Probiotic use is highly prevalent in those with GI conditions (45-60%), and greater compared with those without a GI condition (28%) (Hung et al., 2015, Dossett et al., 2014). Individuals taking probiotics are more likely to be female, have higher educational status and are more likely to be dissatisfied with conventional treatment (Hung et al., 2015). Probiotics are generally considered by gastroenterology patients as a 'natural' avenue of treatment (Hung et al., 2015, Mercer et al., 2012), but familiarity with probiotics is variable and often patients are sceptical about their usefulness and are left with unanswered questions about their role in managing their condition (Mercer et al., 2012). The frequency and pattern of physician-prescribed probiotics in the UK is unknown as large cross-sectional surveys have not been performed. One US survey, however, has found that over 90% of the 56 gastroenterologists

surveyed reported recommending probiotics to patients, and in most cases this was for IBS (Williams et al., 2010).

1.3.2.11 Probiotic as an adjunct therapy

The aim of probiotic supplementation in IBS trials until now has been to investigate their independent effect on reducing GI symptoms. However, there is also an opportunity for the use of probiotic supplementation as adjunct therapy alongside dietary advice. The co-administration of VSL#3, which has demonstrated bifidogenic effects in stool (Brigidi et al., 2003) and mucosal samples (Ng et al., 2013), with dietary advice has never been investigated. Whether this is approach could prevent or at least ameliorate the impact of the low FODMAP diet on the GI microbiota requires investigation. This is especially interesting considering the negative relationship between Bifidobacteria abundance and clinical profile in IBS (Section 1.1.7.4).

1.3.2.12 Conclusion

Probiotic therapy is a safe and patient-acceptable intervention in IBS that has some effectiveness according to RCTs conducted to date. A limited number of viable products are available in the UK, although viability may not be required to induce a clinical effect. Mechanistic data from animal studies has not generally been replicated in human studies, which is likely due to inherent differences in microbiota, diet and stress and coping behaviours. The available evidence suggest effectiveness of probiotic products are strain specific and further robust RCTs are required to confirm the strains or combinations that are most effective for specific IBS symptoms. Their use as an adjunct therapy in IBS has not been evaluated.

1.4 Limitations of dietary research in IBS

The number of RCTs in this field of gastroenterology confirms the increasing interest in the management of IBS symptoms using dietary intervention. However, dietary research is inherently difficult and double-blind placebo-controlled trials, the gold standard method for evaluation of interventions, are almost impossible to conduct when altering whole diets. Furthermore, there is a lack of formal guidance for design and reporting of dietary studies, in contrast to pharmaceutical interventions for which multiple guidelines exist.

Assessment of the effect of broad dietary changes (e.g. as for the low FODMAP diet), as opposed to modification of a specific nutrient or supplementation with a probiotic, is difficult

in IBS for a number of reasons. Firstly, the effect of collinearity applies meaning attributing effects to one component may be flawed (Freudenheim, 1999). For example, restriction of fructans from wheat inevitably leads to reduced gluten intake, which might in itself lead to symptom improvement. Secondly, baseline dietary intake and food beliefs might bias the GI response to dietary intervention. Many patients with IBS hold firm beliefs about food and this might negatively impact patient behaviour in a clinical trial setting (Kramer and Shapiro, 1984).

Other difficulties in dietary research relate to selection bias, blinding and compliance. Complex psychological factors are associated with the desire to be involved in research, and dietary methods for managing symptoms are particularly attractive in IBS. Motivation and behaviours in volunteers may be different to those who do not volunteer for research, or decline involvement in research, and consequent selection bias may affect external validity (Kramer and Shapiro, 1984).

Blinding is difficult in IBS trials as unblinding can occur in response to symptom improvement, and this can lead to a profound placebo effect which can overestimate true efficacy (Kramer and Shapiro, 1984). Probiotic supplementation studies can of course be placebo-controlled, and controlled feeding studies can be placebo-controlled and can help to limit unblinding in whole diet intervention studies, however they do not reflect 'real life' eating behaviour, and the problem of unblinding in response to symptom improvement remains. Nevertheless, measurement of potential bias related to unblinding can be performed by asking participants to guess their allocation (Yao et al., 2013).

Participant compliance with altering their dietary intake is another difficulty in this area of research, and it is generally suboptimally reported, in part due to the difficulty in its accurate measurement. Biomarkers are not available for assessing compliance to whole diets, and therefore indirect measures of compliance are the method of choice (e.g. food diary, questionnaire). There will always be difficulties with conducting robust dietary intervention trials compared with pharmacological trials, and many of these are impossible to avoid. However, it is imperative that future dietary intervention trials continue to attempt to prevent unblinding and measure compliance where possible, and acknowledge the difficulties in doing so.

1.5 Conclusion and future research

Individuals with IBS and other FBD have historically been difficult to treat by both medical and dietary means. The low FODMAP diet for the dietary management of IBS has been of major interest and has helped to successfully treat symptoms in a large proportion of patients. However, further work is needed to confirm the role of the low FODMAP diet, probiotic supplementation or a combination approach in patients with IBS. Whether the impact on stool Bifidobacteria in response to a low FODMAP diet is preventable by use of co-administered probiotic, and indeed if this has an additive effect on symptoms needs to be investigated. The low FODMAP diet is complex to follow and there has been limited work evaluating its effect on HRQOL, nutrient intake or on patient-centered outcomes such as acceptability of advice provided. Finally, the relative importance of host factors in determining response to the low FODMAP diet requires investigation.

1.6 Aims of thesis

1. To design a novel sham diet for use in a blinded placebo-controlled low FODMAP dietary advice RCT in patients with IBS (Chapter 3)
2. To design and undertake a 2x2 factorial design RCT in patients with IBS to investigate:
    a. The effect of low FODMAP dietary advice on IBS symptoms, stool output and HRQOL (Chapter 4)
    b. The effect of low FODMAP dietary advice and probiotic supplementation on the GI microbiota and markers of fermentation (Chapter 6)
    c. The effect of low FODMAP dietary advice on nutrient intake (Chapter 5)

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2 Design and methods of a 2x2 factorial design randomised controlled trial investigating the effect of low FODMAP dietary advice and probiotic supplementation in irritable bowel syndrome

2.1 Introduction

Irritable bowel syndrome (IBS) is a highly prevalent chronic GI disorder (Lovell and Ford, 2012) that contributes to 30% of general practitioner consultations related to GI complaints (Thompson et al., 2000). The economic impact of IBS is considerable (Canavan et al., 2014), and is likely due to its prevalence, chronicity and possibly suboptimal treatment. Historically, the evidence for dietary management of IBS symptoms has been unconvincing. The low FODMAP diet, however, is a promising prospect for symptom management in over two thirds of patients (Staudacher et al., 2014).

A number of limitations exist in RCTs that report evidence for the low FODMAP diet, some of which are inherently problematic in dietary intervention research, some of which were described in Section 1.2.5 and Section 1.4. For example, there is lack of blinding (Staudacher et al., 2012, Pedersen et al., 2014) and variable quality of control groups (habitual diet, alternative dietary advice), comparator dietary advice can overlap with low FODMAP advice (Bohn et al., 2015), and symptoms are evaluated as a secondary outcome (Staudacher et al., 2012). Moreover, there has never been a direct comparison of the effect of low FODMAP dietary advice compared with placebo dietary advice on GI symptoms. There are two reasons why this is required. Firstly, dietary advice as an intervention more precisely reflects routine clinical practice compared with feeding studies, and therefore provides a better estimate of the effect size that could be reliably expected in a clinical situation. Secondly, there is a significant placebo effect in IBS (Elsenbruch and Enck, 2015). Therefore, the effect of low FODMAP dietary advice over placebo advice, rather than habitual diet or standard advice, is a more precise estimate of the independent effect of the low FODMAP diet on IBS symptoms, above and beyond aspects of the consultation, dietetic support and change in lifestyle *per se*.

The effect of the low FODMAP diet on the GI microbiota is a critical area for further research. Preliminary studies demonstrate alterations in Bifidobacteria and other bacterial groups in response to 3-4 weeks of dietary restriction of FODMAPs. However, some of these findings may be limited by sample preparation (Halmos et al., 2015) or the quantification technique used (Staudacher et al., 2012), and further work employing robust methodology is required to broaden our understanding of the response of the microbiota to the low FODMAP diet. Whether any short term microbiota alterations can be prevented using an additional intervention, such as a probiotic, requires exploration. Various other important endpoints

require further examination, including the impact of the low FODMAP diet on aspects of HRQOL which has received limited exploration, and the effect on nutrient intake, and in particular micronutrient intake which has only been evaluated in one small study (Staudacher et al., 2012).

Therefore, a placebo-controlled RCT was designed to address these research questions and build on previous literature. Where possible, a gold standard validated instrument was used for evaluating each endpoint. The following chapter describes the aims of the RCT, the hypothesis to be investigated, and the trial design, protocol, and methods of measurement used to evaluate the endpoints, according to the CONSORT guidelines for reporting RCTs (Moher et al., 2010). A rationale for the use of each method of measurement is provided.

2.2 Aims of the RCT

1. To investigate the effect of low FODMAP dietary advice on adequate relief of GI symptoms in patients with IBS (Chapter 4)
2. To investigate the effect of low FODMAP dietary advice and probiotic supplementation on luminal Bifidobacteria concentration in patients with IBS (Chapter 6)

Secondary objectives:

To investigate the effect of:

1. Low FODMAP dietary advice on IBS symptoms (incidence and severity of specific symptoms), stool output (stool consistency and frequency) and HRQOL (generic and IBS-specific) (Chapter 4)
2. Low FODMAP dietary advice and probiotic supplementation on GI microbiota (total and individual microbiota concentration) (Chapter 6)
3. Low FODMAP dietary advice and probiotic supplementation on markers of fermentation (stool SCFA and pH) (Chapter 6)
4. Low FODMAP dietary advice on nutrient intake (macronutrients, micronutrients, FODMAPs) (Chapter 5)

Hypothesis

This RCT was designed to test two hypotheses:

1. H_1: There is a difference in the proportion of patients reporting adequate relief of IBS symptoms between patients following low FODMAP dietary advice for four weeks compared with patients following placebo sham dietary advice
2. H_1: There is a difference in stool Bifidobacteria concentration between patients following low FODMAP dietary advice and taking a probiotic food supplement compared with patients following low FODMAP dietary advice alone

2.3 The interventions, trial design and approvals

2.3.1 Interventions

Two interventions were employed in this RCT and each was matched with a placebo control.

2.3.1.1 Diet

The dietary intervention, the low FODMAP diet, requires avoidance of fructans, GOS, polyols, fructose and lactose, as described in Section 1.2. The placebo control comparison was a sham diet, a novel exclusion diet developed for this RCT with demonstrated feasibility and dietary composition (Chapter 3). HS assessed baseline dietary intake and provided dietary advice to all patients in the RCT except for a small proportion of patients recruited at the second site. Dietary assessment was undertaken and dietary advice was provided for all patients over the same duration of time (approximately 20 minutes for assessment and 10 minutes for advice). Written dietary information was provided (Appendix 9.1) alongside verbal advice.

2.3.1.2 Probiotic

A number of probiotic products were considered for this RCT. Products available with established viability in the GI tract were *B. lactis* DN-173-010 (Activia), *L. casei* DN 114 001 (Actimel), *B. infantis* 35624 (Align), *L. casei* Shirota (Yakult) or a Bifidobacteria and Lactobacillus multispecies product (VSL #3). A Bifidobacteria-containing product was required at the time of the design of the RCT as current evidence suggested the abundance of this genus was reduced in response to low FODMAP dietary advice (Staudacher et al., 2012). The probiotic that included Bifidobacteria (i.e. unlike Yakult), would not alter nutrient intake (i.e. not in yoghurt such as Activia) and was available in the UK (i.e. unlike Align which at the time was not available in the UK) was VSL#3.

VSL#3 is a probiotic multispecies preparation considered a food supplement within the definition of Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. The active product was supplied in 4.4 g sachets containing 450 billion live bacteria per sachet with maltose and silicon dioxide as inactive excipients. The sachets were gluten free and contained traces of lactose, but were suitable for a low FODMAP diet. The product contained the following eight bacterial species:

- *S. thermophilus*
- *B. breve*, *B. longum*, *B. infantis*
- *L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii* subsp*. bulgaricus*

The placebo was identical to the probiotic in appearance and taste, contained the same inactive excipients but no bacteria, and was administered in the same way as the active product. Probiotic and placebo sachets were provided by VSL Pharmaceuticals. Patients were supplied two boxes (**Figure 2.1**) each containing 30 probiotic/placebo sachets at the beginning of the RCT and were instructed to consume 2 sachets daily, by adding to cold food or fluid at breakfast.

Figure 2.1 Example blinded VSL#3 probiotic/placebo sachets and labelled product box

2.3.2 Trial design

Two trial design options are available for testing a two-fold hypothesis such as in this RCT. Two concurrent parallel arm trials could be conducted, with each testing one hypothesis. Alternatively, a factorial design trial could be conducted. A 2x2 factorial design is the simplest of factorial designs and enables investigation of two interventions individually and in combination. There are four groups, with one group receiving intervention A, one group

receiving intervention B, one group receiving both, and one group receiving neither (**Table 2.1**).

There are two advantages of a 2x2 factorial design study over a parallel arm alternative. Firstly, it promotes a more homogenous cohort as all recruited patients are randomised to the same trial. Secondly, it is more efficient, with a reduced sample size requirement of 70% of an equivalent parallel arm design trial (Mdege et al., 2014). These advantages are based on an assumption that there is a lack of interaction between the two interventions, or that the effect of each treatment is independent of the other. Here the analysis can be described as occurring 'at the margins', and the effect of each treatment is assumed to be additive (McAlister et al., 2003). The presence of an interaction implies that the effect of an intervention is dependent on the effect of another and therefore together they are either synergistic or antagonistic, and the analysis is performed 'within the table' (McAlister et al., 2003).

Table 2.1 2x2 factorial design intervention matrix for the RCT

		Dietary intervention (A)		
		No (sham diet)	Yes (low FODMAP diet)	Margin
Product intervention (B)	No (placebo)	n_o	n_A	N_o
	Yes (probiotic)	n_B	n_{AB}	N_B
	Margin	N_o	N_A	N

A 2x2 factorial design was employed in this RCT with the *a priori* hypothesis that there would be no interaction between the interventions, due to the absence of published evidence to suggest this would be the case for symptom or microbiota endpoints. The anticipated additive outcomes of the interventions on the microbiota endpoint are described in **Figure 2.2**.

The RCT was designed to measure outcomes in response to a 4-week intervention (**Figure 2.3**). Four weeks was considered a sufficient duration for evaluation of endpoints. It is known that diet-induced microbiota alterations can occur very rapidly, even within 2 days (David et al., 2014), but trials assessing symptom response to a low FODMAP diet are usually of 3-4 weeks' duration (Halmos et al., 2014, Staudacher et al., 2012, Bohn et al., 2015), and this is the minimum intervention duration recommended for IBS trials (Irvine et al., 2006). Furthermore, a 4-week intervention period allows measurement of outcomes at approximately the same

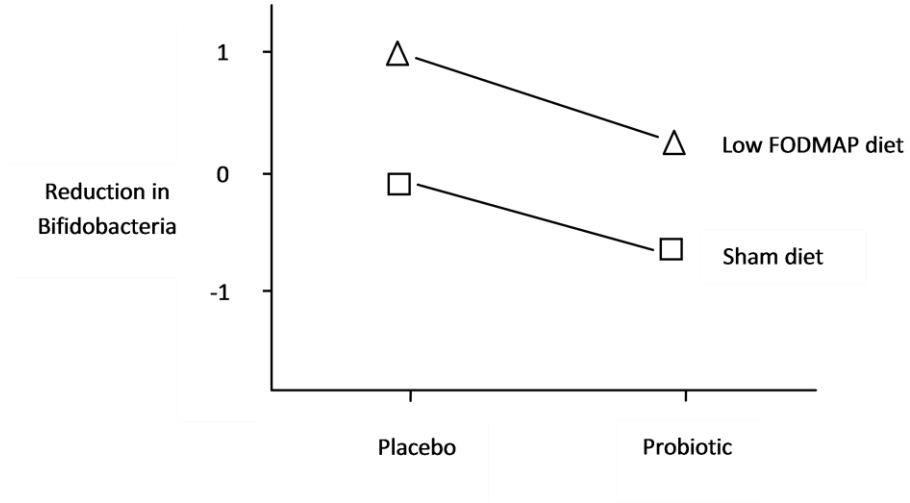

Figure 2.2 Estimated anticipated outcome of the interventions on Bifidobacteria concentration in the RCT. The parallel lines indicate the effect of the dietary intervention is independent of the effect of the probiotic

stage of the menstrual cycle at both timepoints in females. This reduces the confounding effect of the menstrual cycle, which has been reported to influence symptoms in IBS (Heitkemper et al., 1995).

2.3.3 Trial sites

Patients were recruited from Gastroenterology and Dietetic Outpatient Clinics at Guy's and St Thomas' NHS Foundation Trust and St George's Healthcare NHS Trust. The trial visits took place at King's College London, School of Medicine, Diabetes and Nutritional Sciences Division, London, for Guy's and St Thomas' patients and at St George's Hospital, London, for St George's patients.

2.3.4 Patient selection

Patients were recruited from Gastroenterology and Dietetic Outpatient Clinics between January 2012 and November 2014. Patients were not recruited during December months to avoid poor compliance during the festive period and the confounding effects of alterations in diet on the microbiota (Gougoulias et al., 2008). Patients were required to fulfil the following criteria:

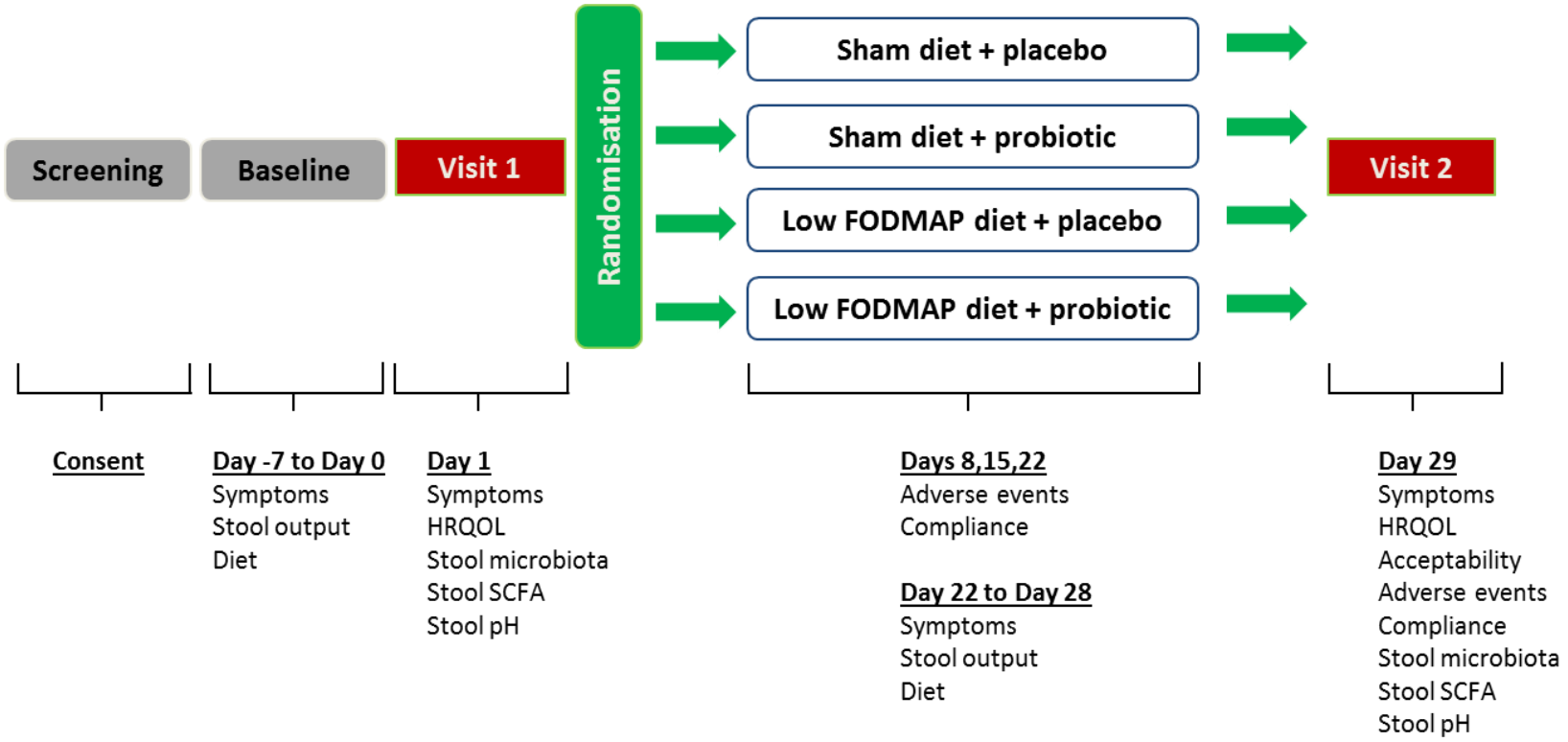

Figure 2.3 RCT design

2.3.4.1 Inclusion criteria

- Males and females aged 18-65 years with IBS-D, IBS-M or IBS-U according to Rome III criteria who do not have a major medical condition (e.g. diabetes, major active psychiatric condition), GI disease (e.g. IBD, coeliac disease) or history of GI resection. IBS-C patients were not included due to a lack of evidence for the effectiveness of the low FODMAP diet in this IBS subtype
- Individuals able to provide informed consent
- Individuals naïve to a low FODMAP diet

2.3.4.2 Exclusion criteria

- Abdominal pain or discomfort for less than 2 days in the screening week as recommended for trial eligibility screening (Longstreth et al., 2006)
- Females who report to be pregnant or lactating, as this may impact on GI symptoms (Carlin and Alfirevic, 2008)
- Consumption of antibiotics, prebiotics or probiotics (as supplemented to food products or as supplements) in the 4 weeks prior to, or during the RCT as this influences the microbiota
- Use of lactulose or orlistat as they may lead to altered GI fermentation, and change to medications that might impact on GI function (e.g. biological therapy, cathartics)
- Patients who have received bowel preparation for investigative procedures in the previous 4 weeks prior to or during the RCT as this influences the GI microbiota (Jalanka et al., 2015)
- Patients who travelled to a developing country in the previous 2 weeks, as presence of GI infection may contribute to symptoms and/or influence the GI microbiota
- Changes to IBS medication or dose in the 4 weeks prior to or during the RCT as this may affect IBS symptoms
- Patients with additional specific dietary needs (e.g. iron deficiency, eating disorder, significant dietary restrictions such as gluten free/dairy free) as further dietary restriction may compromise nutritional status
- Lactulose or glucose hydrogen breath test less than 2 weeks prior to screening or during the RCT as this could impact on symptoms, or lactose and/or fructose breath tests within the last 6 months or during the RCT, as this might stimulate interest in aspects of the low FODMAP diet and lead to unblinding

2.3.5 Sample size calculation

The sample size calculation was based on co-primary endpoints of adequate relief of symptoms and stool Bifidobacteria concentration and it was assumed that there was no interaction between the main effects. Expected outcomes used for the calculations are presented in **Table 2.2**. The sample size calculation was performed by HS and Professor Kevin Whelan in consultation with Professor Janet Peacock (Professor of Medical Statistics) using simulation in the statistical software package 'R'. The sample size calculation was based on previous data as follows:

Hypothesis 1: The estimation of adequate relief of symptoms for the low FODMAP diet was based on the proportion of patients reporting adequate relief after low FODMAP advice from the only RCT available at the time (Staudacher et al., 2012). Combined global response data for placebo and probiotic supplementation from VSL#3 supplementation studies (Kim et al., 2005, Kim et al., 2003) was used for placebo and probiotic estimates. The estimated symptom response to sham diet was based on placebo response rate for global symptoms in the largest meta-analyses of IBS trials at the time (Pitz et al., 2005). It also represented the approximate midpoint of proportion of control patients reporting symptom relief following habitual diet in a previous unblinded controlled low FODMAP advice RCT (Staudacher et al., 2012) and controls reporting symptom relief in a comparison of low FODMAP advice with standard dietary intervention (Staudacher et al., 2011), and therefore was considered representative of symptom response to a placebo dietary intervention.

Hypothesis 2: Expected stool Bifidobacteria concentration in response to a low FODMAP diet was available from one RCT published at the time, and sham and placebo groups were estimated to harbor Bifidobacteria concentration equivalent to controls from that study (Staudacher et al., 2012). Increased total Bifidobacteria concentration has been demonstrated after short term VSL#3 supplementation (Brigidi et al., 2003), which was summed with the placebo value to produce the expected mean Bifidobacteria concentration for the probiotic group. Expected standard deviations (SD) were estimated from previous data (Staudacher et al., 2012).

Based on logistic regression and assuming a power of 80% and a 2-sided significance level of 5%, the main effects of diet on adequate relief could be estimated with 88 patients. Based on previous work from our group (Staudacher et al., 2012), attrition of 12% was estimated,

Table 2.2 Expected outcomes for primary endpoints

Group	Proportion of patients reporting adequate relief of GI symptoms (%)	Bifidobacteria concentration Mean (SD) (log_{10} cells/g faeces)
Sham diet	36	8.2 (0.6)
Low FODMAP diet	68	7.4 (0.7)
Placebo	35	8.2 (0.6)
Probiotic	42	9.2 (0.7)

leading to an overall sample size of 100 allowing for attrition. The RCT required a sample size of 1572 patients to detect the effect of probiotic on adequate relief which was not feasible in the current time frame. Therefore the study was underpowered to detect the effect of the probiotic on adequate relief.

Based on linear regression and assumed power 80% and overall 2-sided significance 5%, the main effects for the Bifidobacteria outcome could be estimated with 24 patients, or 28 allowing for attrition. Therefore, the study was powered for both outcomes with an overall sample size of 100 allowing for attrition.

2.3.6 Recruitment

Patients were recruited at Guy's and St Thomas' NHS Foundation Trust by HS (n=96). A research assistant assisted with screening for 3 months midway through the 2-year recruitment period. HS and RN (a dietitian) recruited patients at St George's Hospital (n=8). HS promoted awareness of the RCT to the referring clinicians (gastroenterologists, general practitioners) through presentations at team meetings and regular email contact. Gastroenterologists were requested to notify patients regarding the RCT in clinic, and a referrer 'leaderboard' within the Guy's and St Thomas' Gastroenterology department was set up by HS to promote ongoing participation of clinicians in referring patients to the RCT.

2.3.7 Randomisation

Block randomisation was used to allocate patients to one of the four treatment groups (**Figure 2.3**). Randomisation was stratified by gender and the presence of IBS-D as microbiota composition may be different in this IBS subtype (Malinen et al., 2005). A random allocation sequence was prepared by researchers not involved in patient screening or recruitment

(Professor Kevin Whelan, Dr Miranda Lomer) using a random number generator (Microsoft Excel, 2010), and was stored securely in a location that was inaccessible to the clinic room used for trial visits. Allocation was by a 1:1 ratio with a block size of 8 to ensure even numbers of patients across the four groups throughout the study. The block size was blinded to HS and RN who recruited patients and conducted randomisation visits.

2.3.8 Blinding

Blinding of dietary interventions is rarely effective (Yao et al., 2013), hindering the ability to administer a successful blinded placebo diet. However, the fact that both the dietary and probiotic interventions were blinded to patients in this RCT may have mitigated some of these problems (as if one intervention became unblinded the patient was still blinded to the other). Nevertheless, it is suggested blinding success be measured and reported (Moher et al., 2010), but interpreted in the context that methodological issues of placebo and blinding are a particular challenge in dietary intervention studies.

Patients were blinded to the low FODMAP/sham diet and probiotic/placebo allocation. The patient information sheet (Appendix 9.2) did not mention the term 'low FODMAP', instead referring to an intervention that 'alters the carbohydrates in your diet', in order to prevent patients familiarising themselves with the diet. The researchers providing the advice (HS, RN) were specialist dietitians with expertise in the low FODMAP diet, and could not be blinded to the dietary allocation. Opaque envelopes labelled with sequential participant numbers concealed patients' dietary allocation until all baseline data had been collected at the randomisation visit.

The probiotic intervention was blinded to the patients and the researcher conducting the trial visits (HS, RN). Researchers who were not involved in patient recruitment or patient visits (Dr Miranda Lomer, Lee Martin) labeled batches of product prior to commencement of the RCT with participant numbers according to the randomisation sequence. Pre-labeled product was provided to patients at the end of the randomisation visit. Product boxes and sachets were identical in appearance and were identifiable only by participant number. Patients were unblinded to the dietary allocation at the end of Visit 2 and were notified regarding probiotic/placebo allocation after data analysis and unblinding.

2.3.9 Compliance

Clinical observational studies and case note reviews suggest proportions of patients that comply with dietary advice varies substantially (13-82%) (Glanz, 1980), and 76% of patients in dietary research report themselves to be compliant (Crumb-Johnson et al., 1993), although the definition of compliance and its method of measurement is inconsistent between studies. Compliance with dietary advice is difficult to measure in the absence of valid biomarkers, and there is no standardised tool for measuring dietary compliance to the low FODMAP diet. Therefore, compliance to dietary advice is usually measured by patient self-report. Previous studies have used measures of dietary compliance such as the proportion of days the patient reports adherence to the recommendations provided (Mitchell et al., 2011, Halmos et al., 2014), frequency of consumption of unsuitable foods (Windhauser et al., 1999, Halmos et al., 2014) and adherence based on pre-defined categories (Shepherd and Gibson, 2006).

In this RCT, dietary compliance was measured using two methods. It was objectively measured by post hoc assessment of FODMAP intake at the interim analysis (Section 3.4.2) and at final dietary analysis (Section 5.2.3). Self-reported compliance was also assessed at weekly telephone visits using four categories ('in the last week I have followed the diet never/rarely (<25% of the time), sometimes (25-50% of the time), frequently (51-75% of the time) or always (76-100% of the time)) adapted from previous work (Shepherd and Gibson, 2006). A list of five unsuitable foods, based on the patient's usual dietary intake, was used as a prompt at the weekly phone calls to increase accuracy of responses. For this RCT, noncompliance was defined by self-report because there is no objective threshold FODMAP intake value that defines a diet as 'low FODMAP'. Patients who reported following the diet <50% of the time on at least 2 of the 4 assessments were considered noncompliant to the dietary intervention.

Measurement of compliance is remarkably infrequently reported in probiotic supplementation trials. Inspection of unused product is commonly used to measure compliance, where compliance is defined as consumption of at least 80% of product provided (Simren et al., 2010). Evaluation of the microbiota in those patients allocated probiotic has also been used as a measure of compliance. Whether there is a compliance threshold below which microbiota and/or symptoms are unaffected by probiotic is unknown. Therefore, in this RCT compliance with the probiotic/placebo was considered intake of product on at least 80% of the trial duration in concordance with previous trials, by counting of unused sachets at Visit 2 and assessment of the compliance diary (Appendix 9.3). It was also deemed noncompliant if

compliance fell below 80% due to a recommended reduction in dose due to perceived side effects (Section 2.3.10).

2.3.10 Adverse events and withdrawals

Adverse events were recorded at each visit and at weekly telephone contacts. GI symptoms were only recorded as adverse events if they emerged or worsened relative to baseline. Duration, frequency and intensity of the adverse event, medication usage and possible relationship with probiotic/placebo were recorded. For each individual, multiple reports of adverse events were counted once and repetitions ignored. Where adverse events were thought to be due to the probiotic/placebo product, the patient was advised to split the dose (i.e. 1 sachet in the morning, 1 sachet in the evening). If after 3 days the symptoms continued, the patient was advised to reduce the dose to 1 sachet per day. In the case of a serious adverse event, it would have been reported to the Research Ethics Committee and the relevant Research and Development office.

Patients were free to withdraw from the RCT at any time, according to standard ethics protocol. Patients who wished to discontinue participation in the RCT were classified as 'consent withdrawal', or were identified as a 'loss to follow up' if the patient was uncontactable and subsequently did not complete the trial. Furthermore, patients were withdrawn if they failed to meet the eligibility criteria during the RCT (e.g. commenced antibiotic therapy).

2.3.11 Ethics approval and Good Clinical Practice

Ethical approval for this RCT was granted on 12 October, 2012 by the National Research Ethics Service Committee London Fulham (12/LO/1402). Research and Development approval was granted by King's College London on 9 November, 2012 and by St George's Healthcare NHS Trust on 10 January, 2013. The RCT was registered on the ISRCTN registry on 28 August, 2012 (ISCRCTN02275221). The lead researcher completed Good Clinical Practice and Human Tissue Act and Consent Training prior to recruitment and processing of samples.

The RCT was conducted in compliance with the principles of the Declaration of Helsinki (1996), and the principles of Good Clinical Practice. There were no major ethical risks associated with participation. The only potential burden for patients was the embarrassment associated with collecting stool samples, and the requirement for recording dietary and symptom information,

the latter of which is part of standard clinical practice. All patients who were allocated to the sham dietary intervention were provided with routine clinical dietary advice (including low FODMAP dietary advice) at the final visit it was clinically warranted, but this was only performed following all outcome data measurement and unblinding. There was no delay in standard clinical intervention for trial patients compared with equivalent patients on the routine clinic waiting list.

The reasons for the research and the trial procedures were clearly explained and provided in a participant information sheet to eligible patients interested in participation (Appendix 9.2). Patients were given at least 24 hours to consider participation and ask further questions about the research. Written informed consent was obtained prior to any trial procedures (Appendix 9.4). Patient details were anonymised by allocation of a screening number on screening and a participant number on randomisation. All personal data and research data were stored securely at King's College London. Electronic data were password protected and hard copy data were stored securely in a locked filing cabinet.

2.4 Trial protocol and procedures

Standardised trial data documents were used at each stage of the recruitment and data collection process to ensure uniformity of practice and completeness of data collection.

2.4.1 Screening

Screening was conducted in two parts. Patients were identified as potentially suitable from outpatient clinics or referral letters. Medical notes were screened and if the patient appeared eligible, they were contacted by telephone. If the patient was interested in participating, they were screened against the inclusion and exclusion criteria and a brief diet history was taken to assess current dietary restrictions and to evaluate whether the patient was naïve to the low FODMAP diet. Once this had been completed, a participant information sheet was sent to the patient. After at least 24 hours, the patient was contacted and a consent visit was booked if they were interested in participating.

At the consent visit, the trial procedures were clarified, the consent form was signed, a copy was filed at the research site and a copy was provided to the patient. A symptom, stool and diet record (Appendix 9.5) and stool collection kit were provided. The symptom record included a daily Gastrointestinal Symptom Rating Scale questionnaire (Section 2.5.1.3) and the

adequate relief question (Section 2.5.1.1) on day 7. The stool record required daily stool frequency and consistency recording using the BSFS (Section 2.5.1.4). Patients were advised on the method of stool collection, and the importance of the timing of stool collection at Visit 1 (Section 2.4.2). The method of stool collection was described verbally and written information was provided (Appendix 9.6). Patients were advised that all smoking, dietary habits and medication were to be maintained during screening. Visit 1 was tentatively booked at a time of day that the patient was confident they could provide a stool sample, pending successful completion of the second stage of screening.

The second stage of screening involved completion of the 7-day symptom, stool and diet record. This evaluated whether patients met symptom severity criteria (Section 2.3.4.2) and also served as baseline data for those who were subsequently eligible. Final eligibility was confirmed prior to Visit 1.

2.4.2 Visit 1

At Visit 1 the remaining baseline data were collected. The baseline symptom, stool and diet record was returned. Care was taken to ensure all symptom data were complete. Ambiguity in the diet records was clarified (Section 2.7.2). The order of data collection was kept consistent to minimise variability in response bias between patients. The following data were collected:

- Current medications, past medical history, smoking history and duration of IBS symptoms
- Anthropometry (height, weight, body mass index)
- IBS-SSS questionnaire (Section 2.5.1.2, Appendix 9.7)
- SF-36 and IBS-QOL questionnaires (Sections 2.5.2.1 and 2.5.2.2 and Appendices 9.8, 9.9)

A fresh stool sample was collected during the visit, or no longer than one hour prior to Visit 1 and immediately placed on ice. At the conclusion of baseline data collection, the randomisation envelope was opened, the patient was randomised to the low FODMAP/sham diet, dietary advice was delivered and the written dietary information provided. Probiotic/placebo product was allocated in coded boxes, with instructions on frequency and timing of administration. A probiotic/placebo compliance diary (Appendix 9.3) and a final symptom, stool and diet record was provided for completion in the week prior to Visit 2. Weekly monitoring phone calls and Visit 2 dates were booked and contact details for the research team were provided.

2.4.3 Weekly monitoring

Patients were contacted once a week during the intervention period by telephone. This served a number of purposes. Importantly, patients were prompted to adhere to the dietary advice and with taking the probiotic/placebo product. This was carried out after self-reported compliance to the diet, adverse events, medication usage and smoking habits were recorded. Finally, the call served as an opportunity for patients to ask questions about the diet. Brief clarification was provided where required. At the final weekly telephone contact (week 3), patients were reminded to commence recording in the symptom, stool and diet record, and to return the record and unused probiotic/placebo product sachets at Visit 2.

2.4.4 Visit 2

At Visit 2 all follow up data were collected. The follow up symptom, stool and diet record was collected. As per Visit 1, the order of data collection was kept consistent between patients. The data collected, in order, included:

- Current medications and smoking history
- Anthropometry (weight, body mass index)
- IBS-SSS questionnaire
- SF-36 and IBS-QOL questionnaires
- Diet/probiotic acceptability questionnaire (Section 2.8, Appendix 9.10)
- Unused placebo/probiotic product sachets were counted and compliance diary assessed
- Success of blinding was measured by asking patients to guess their diet and probiotic/placebo product group allocation

A fresh stool sample was collected during the visit, or no longer than one hour prior to the visit and immediately placed on ice. Once all data had been collected, those patients requiring further dietary advice (e.g. low FODMAP advice for those in the sham diet group) were advised as appropriate and all patients were informed of the name of the probiotic product under investigation.

2.5 Methods of measurement and rationale: Clinical effectiveness

2.5.1 Symptoms and stool output

The symptom experience of patients with IBS is multi-dimensional and can be categorised into abdominal, defecatory and extraintestinal domains (Spiegel et al., 2010a). Measurement of symptom response in IBS should therefore span each of these domains. The three methods by which symptoms can be assessed in IBS is via clinical interview, provocation tests or subjective reporting. Clinical interview is time consuming and may underestimate symptom severity (McColl, 2004) and the usefulness and validity of provocation tests (e.g. rectal barostat) has been questioned (Mujagic et al., 2015). Subjective reporting is the most appropriate means of measuring symptom experience (McColl, 2004) and there are a number of validated questionnaires for measuring GI symptoms in IBS. Use of a questionnaire in isolation may be problematic as most are considered suboptimal (Trentacosti et al., 2010) and they are subject to recall bias. Symptom diaries, however, are considered the gold standard method for data collection of this type, are less subject to recall bias (McColl, 2004), and allow for measurement of incidence and fluctuation of severity of symptoms over time.

Electronic recording of symptoms is preferred over paper diaries, as it prevents retrospective recording (McColl, 2004). Indeed, an electronic diary fitted to a watch band was tested (PRO-Diary Version 1.0.29) for this RCT. Cost (£800 for 2 units) and the restricted screen word count per question were limiting factors. Furthermore, paper diaries are less burdensome for those patients who are less technologically capable. In light of the above, and to capture a broad dimension of symptom experience from abdominal, defecatory and extraintestinal domains, prospective diary recording and a validated IBS symptom questionnaire were chosen for this RCT. Face to face instruction was provided for completion of both, with telephone and email reminders where required to improve completion rate (McColl, 2004).

2.5.1.1 Rationale for choice of method: Adequate relief

The current gold standard for primary outcome assessment in treatment trials for IBS is the dichotomous response global symptom question, 'do you have adequate relief of your IBS symptoms?' (Irvine et al., 2006). Global symptom measures such as this have good correspondence with specific GI symptoms (Irvine et al., 2006). However, there are a number of limitations of this as an evaluation of symptom response. Firstly, a binary response doesn't allow for detail regarding clinically important individual symptoms, which is better captured by a multi-item instrument. Secondly, it does not enable reporting of symptom exacerbation.

Thirdly, if there has been improvement, a quantification of the magnitude of the improvement is impossible. Fourthly, although it appears a simple question, it is demanding for patients to answer, as it requires synthesis of one response in relation to a multitude of individual symptoms (McColl, 2004), and the frame of reference for what is 'adequate relief' is probably variable between patients.

Another proposed flaw of the adequate relief question is that is potentially confounded by baseline symptom severity. Specifically, it is reported to be biased towards patients with less severe IBS, who are more likely to have adequate relief in response to treatment, in the absence of improvement of other measures (Whitehead et al., 2006, Passos et al., 2009). There is some debate about this, however, and one subsequent study pooled data from a total of 9000 IBS patients from 12 RCTs and found no association between adequate relief and baseline severity (Spiegel et al., 2009). The adequate relief question is easy to administer, is clinically and statistically relevant in IBS drug trials, and has demonstrated content and criterion validity (Irvine et al., 2006). Many of its limitations can be addressed by administration of additional tools, and therefore this 'gold standard' measure was selected for use in this RCT and was administered on day seven of the symptom and stool recording period at baseline and follow up (Appendix 9.5).

2.5.1.2 Rationale for choice of method: IBS Severity Scoring System (IBS-SSS)

Multi-item tools that assess specific symptoms are of benefit where physiological processes are hypothesised to be associated with certain symptoms (Naliboff et al., 1999). The IBS-SSS is a 2-part questionnaire designed to assess individual symptoms over the previous 10 days. It has demonstrated concurrent validity, sensitivity and reliability (Francis et al., 1997). The first part of the questionnaire includes five VAS items evaluating abdominal pain frequency and intensity, abdominal distension, satisfaction with bowel habit, and quality of life (Appendix 9.7). The second part collects information on bowel habit and site of pain, but is not scored and data generated here is usually not reported in the literature.

Although VAS are limited by a lack of consistent meaning between individuals regarding a specific point on the scale (Wyrwich and Tardino, 2004), they provide continuous data which is simpler for analysis purposes, and have been shown to be validated for measurement of pain (Naliboff et al., 1999). Furthermore, with regard to the IBS-SSS, scoring allows categorisation of patients into mild (75-174), moderate (175-300) and severe (>300) cases, with a maximum

achievable severity score of 500 points, which is valuable in IBS where formal severity categories are not established (Drossman et al., 2011). Moreover, a minimal clinically important difference (MCID) of a 50-point reduction in score allows meaningful interpretation of score change over time (Francis et al., 1997).

Although it has a number of merits, there are some limitations of the IBS-SSS. A major contribution to the total IBS-SSS score comes from questions regarding pain intensity and incidence. Although abdominal pain is a key feature of IBS, and its intensity and frequency should be captured, abdominal discomfort, flatulence and other symptoms, all perceived as important symptoms by patients (Spiegel et al., 2010b), are not measured in the IBS-SSS. Also, distinguishing abdominal pain from discomfort is difficult for patients (McColl, 2004), which provides opportunity for measurement error. Furthermore, the stool output question ('how satisfied are you with your bowel habit?') may not be sensitive to detect changes in specific symptoms such as urgency or change in stool consistency. Finally, characterising symptoms over a discrete period of time may be difficult for patients, and patients might recollect and report symptoms from over a broader period of time than required (Bellini et al., 2010).

Overall, despite its limitations, the IBS-SSS has been regarded as an important tool for rating symptom severity in IBS and a recent comparative review of patient reported outcomes concluded that it is the preferred questionnaire for obtaining detailed information on symptoms in IBS (Bijkerk et al., 2003). It allows rating of response with a numerical score, classifies IBS severity and identifies those who achieve a MCID, all of which were considered of value for this RCT.

2.5.1.3 Rationale for choice of method: Gastrointestinal Symptom Rating Scale (GSRS)

The GSRS is a comprehensive symptom questionnaire initially developed and assessed for reliability in patients with IBS and peptic ulcer disease (Svedlund et al., 1988). GI symptoms and stool output are rated on a Likert scale, which initially included limited descriptors of severity, frequency, duration and impact on life. The scale has been validated in patients with gastroesophageal reflux disease (Revicki et al., 1998) and IBS (Wiklund et al., 2003).

The benefits of this scale include the ability for it to be used prospectively and daily (Revicki et al., 1998), which is recommended for frequently occurring symptoms (Trentacosti et al., 2010) and minimises recall bias (Irvine et al., 2006). Although the most recent version excluded items

for belching and borborygmi, it assesses the most important IBS symptoms (Wiklund et al., 2003). The GSRS was therefore used as the basis of the 7-day symptom record for this RCT. It was modified to include only items considered important in IBS. Items from the previous version (heartburn, acid reflux, nausea, borborygmi, belching) (Revicki et al., 1998) were added and items considered irrelevant or repetitive (two items relating to feeling full, visible swelling, relief of pain in response to bowel action) were removed. New items relating to tiredness and overall symptoms were also added, with a final scale consisting of 16 items (Appendix 9.5). Patients were asked to score the GSRS at the end of each day for seven days at baseline and at follow up, rather than the entire 4-week period to reduce patient burden (McColl, 2004). A minimum of three days was considered sufficient for data analysis if the record was not complete. The possible responses for each GI symptom were:

0 = absent ('I didn't have this symptom')
1 = mild ('I had it but it didn't bother me much')
2 = moderate ('it bothered me quite a bit')
3 = severe ('it bothered me a great deal')

Summary data for each patient was then calculated:

Incidence = Number of days the symptom was recorded as present during the 7-day recording period (i.e. scores 1-3)

Severity = Average daily severity score (total 7-day severity score divided by 7)

2.5.1.4 Rationale for choice of method: BSFS

Stool frequency and form (consistency) are two important parameters in the measurement of stool output. Characterisation of stool output in community-dwelling individuals reveals there is normal variation in stool frequency in healthy individuals. A majority of individuals pass stool once in 24 hours (33% of women, 40% of males), but most range between a frequency of once every two days to twice a day (Heaton et al., 1992). Although this is not recent data it is the most comprehensive so far. Similarly, stool form is described as formed in a majority of healthy individuals, but up to 27% of females and 19% of males report hard stools (Heaton et al., 1992).

In IBS, altered stool output is required for diagnosis (Longstreth et al., 2006). Therefore, the assessment of both frequency and consistency is important in evaluating response to intervention. Stool frequency recorded prospectively in a daily diary is a better alternative to retrospective evaluation which can be subject to recall bias, particularly for those with diarrhoea or constipation (Lackner et al., 2014). Evaluation of stool consistency should be performed using a diary comprising the BSFS (Longstreth et al., 2006), which is a written and pictorial scale and has been validated in IBS (**Figure 1.2**) (O'Donnell et al., 1990).

There is lack of agreement on what is constituted as 'abnormal' stool consistency, particularly for diarrhoea (Whelan et al., 2003), and therefore the BSFS adds to and is more objective than measures of stool output from the IBS-SSS and GSRS. According to the BSFS, stool is categorised on a 7-point scale based on cohesion of the stool surface. Types 1 and 2 stools on the BSFS are considered indicative of constipation and Types 6 and 7 of diarrhea (Longstreth et al., 2006). As for stool frequency, prospective evaluation of stool consistency is preferred, due to discordance between recall and diary recording (Coletta et al., 2010, Bellini et al., 2010). Therefore, patients in this RCT recorded their stool frequency and consistency using the BSFS for 7 days at baseline and follow up, and were encouraged to record their stool output throughout the day.

2.5.2 HRQOL

Health-related quality of life (HRQOL) is a measure of a patient's experience of health and disease. It is an indication of disease impact (Guyatt et al., 1993), which is especially important in conditions marked by morbidity rather than mortality. Clinical response to a treatment may not necessarily correspond with a comparable response in emotional and physical functioning HRQOL endpoints between patients, and therefore HRQOL is in itself an important measurable outcome in clinical trials (Lea and Whorwell, 2001)

HRQOL can be assessed by face-to-face interview or interviewer- or self-administered questionnaire. Face-to-face interview maximises response rate and leads to minimal missing data but requires an appropriately trained interviewer, whereas validated questionnaires are simple to use and require minimal resources. There has been substantial growth in the number of HRQOL questionnaires available in recent years (Garratt et al., 2002). One disadvantage of HRQOL questionnaires is that patients may misunderstand questions which increases the likelihood of missing data (Guyatt et al., 1993). Supervised completion of questionnaires can

address both of these issues. Most validated HRQOL questionnaires consist of items that contribute to specific domains or scales (e.g. physical functioning, emotional functioning) and a total score.

2.5.2.1 Rationale for choice of method: Short Form Health Survey 36 (SF-36)

Generic HRQOL instruments capture a broad range of HRQOL issues relating to the effect of health status on functional capacity and emotional wellbeing. They can be applied to a variety of patient populations and are useful in a discriminative capacity, but are not especially responsive to change in treatment. There are a number of instruments available (e.g. SF-36, EuroQOL, SIP). The SF-36 is the most evaluated HRQOL questionnaire in health research (Garratt et al., 2002), is commonly utilised in the validation of other HRQOL measures, and was used as the generic HRQOL instrument in this RCT (Appendix 9.8).

The SF-36 consists of 36 items that are either Likert-style response scale items or dichotomous scale questions. It includes more concepts than the original 20-item MOS short-from survey, which demonstrated floor effects (responses form a negatively skewed distribution; (Ware and Sherbourne, 1992) and is based on a response recall period of 4 weeks. Scoring involves transformation of each item to a 0-100 scale and calculation of scores for eight individual domains (physical functioning, role limitations because of physical health problems, bodily pain, social functioning, general mental health, role limitations because of emotional problems, vitality and general health perceptions), with a higher score representative of better HRQOL.

2.5.2.2 Rationale for choice of method: IBS-QOL

Disease-specific HRQOL instruments focus on aspects of the disease of interest and are more responsive to change in a defined cohort (Guyatt et al., 1993). There are a number of such instruments designed to measure IBS-specific HRQOL (e.g. IBS-QOL, DHSI, FDDQOL, IBS-HRQOL). The IBS-QOL is a 34-item instrument with demonstrated reliability and validity in IBS (Patrick et al., 1998, Drossman et al., 2000). It is the most extensively psychometrically tested HRQOL instrument in IBS and recommended as the instrument of choice for this population (Bijkerk et al., 2003), and therefore was employed as the disease-specific HRQOL instrument for this RCT (Appendix 9.9). Each item utilises a 5-point Likert response scale, which is transformed to a 0-100 point scale and subscale scores are then calculated (dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual,

relationships). A total score can be calculated by summing the subscale scores. A score of 100 denotes maximum HRQOL for subscales and the total score, and the MCID is a 14-point reduction in score (Drossman et al., 2007). As for the SF-36, the response recall duration is also four weeks.

2.5.2.3 Questionnaire administration

Patients completed the HRQOL questionnaires immediately following administration of the IBS-SSS questionnaire during the trial visits. The IBS-QOL was administered first and was followed by the SF-36 to reduce response bias. Patients were instructed to take their time and to ask questions if an item was unclear. Patients were reminded to use a recall period of four weeks while completing both questionnaires.

2.6 Methods of measurement and rationale: Microbiota and markers of fermentation

2.6.1 Sample collection

The GI microbiota can be evaluated either using stool samples or colonic mucosal samples. It is acknowledged, however, that these compartments vary in microbiota composition in IBS and in healthy controls (Rangel et al., 2015). Although mucosal samples may be more reflective of GI disease pathophysiology, they are difficult to obtain and bowel preparation prior to colonoscopy may alter the microbiota (Jalanka et al., 2015). Stool samples are limited by alterations in species composition that occur between collection and processing (Fraher et al., 2012). However, historical evaluation of the microbiota in IBS has generally relied on stool samples, and due to the non-invasive nature of sampling, stool samples were collected to characterise the microbiota and evaluate markers of fermentation in this RCT.

Sample collection and preparation in the RCT was standardised to reduce technical variability. Stool was collected by patients at Visit 1 and 2 using the stool collection kit provided. A whole stool was collected into a transparent bag placed over a disposable plate placed into the toilet bowl (Appendix 9.6). Patients were asked to refrain from urinating during voiding. The stool was collected either at the research site during the visit or no more than one hour prior to attending the research site for the scheduled visit. Patients who voided prior to the visit transported stool on ice in sample containers provided. Samples were immediately placed on ice at the research site until processing.

The stool sample was processed immediately after the research visit. Although DNA degrades at room temperature, the minimum time required before significant changes occur to the microbiota and/or the luminal environment (e.g. pH, SCFA) is unclear, but could range between 30 minutes and 14 days (Gorzelak et al., 2015, Lauber et al., 2010). In this RCT, time between storage on ice and freezer storage was limited to no more than 2 hours. The sample was double bagged and homogenised in a stomacher for 4 minutes (Steward Laboratory Blender Stomacher 400). This was required as differences in bacterial taxa are evident between the inner and outer stool microenvironments (Gorzelak et al., 2015). Multiple aliquots were taken for analysis of the microbiota, SCFA and pH and stored at -80°C.

2.6.2 qPCR

qPCR was used to measure the abundance of the stool microbiota in this RCT. This was performed by HS under the supervision of Professor Kevin Whelan, Dr Matthew Arno (Genomics Centre Manager, King's College London) and Dr Petra Louis (Senior Research Fellow, University of Aberdeen).

2.6.2.1 DNA extraction

Extraction of DNA from stool samples is an essential step for both downstream analyses of the GI microbiota. The major steps involved in extraction are disruption and lysis of the bacterial cells usually chemically (by lysis buffer or enzymes) and/or mechanically (e.g. bead beating or sonication), removal of proteins, fat, carbohydrate, RNA and inhibitors (by addition of protease and detergents), and finally, the recovery of DNA (Tan and Yiap, 2009). Commercial kits available to perform extraction utilise different techniques including organic extraction, 'salting out' and silica-based methods. Limitations of extraction that might bias downstream outputs include insufficient lysis or incomplete removal of contaminants. These may occur as a result of an inefficient extraction method or impurity of the reagents (Tan and Yiap, 2009, Ariefdjohan et al., 2010).

Studies comparing DNA extraction techniques have generally compared lysis efficiency and subsequent DNA yield and diversity between methods. Methods that utilise mechanical bead beating better maintain community structure of the human microbiota compared with enzymic lysis methods (Yuan 2012,). Specifically, the FastDNA™ SPIN kit for soil (MP Biomedicals Europe, Illkirch-Graffenstaden, France), which incorporates lysis of cells by bead-beating, has demonstrated superior DNA yield compared with three other extraction kits

(Ariefdjohan et al., 2010). Therefore this kit was used to extract DNA for this RCT. This technique consists of mechanical lysis of bacterial cell walls followed by silica-based separation and purification of DNA using binders and centrifugation. All reagents were provided by the manufacturer. Sample preparation and DNA extraction was performed by HS as follows.

2.6.2.1.1 Sample preparation and storage

Immediately after the trial visit, a 3-5 g aliquot of fresh stool was weighed and diluted 1:3 in phosphate-buffered solution (PBS)/30% glycerol solution, which reduces DNA degradation and ice crystal formation (Squires and Hartsell, 1955). Glass beads were added and samples were vortexed for 2 minutes. Once completely homogenised, approximately 500 µl of the diluted sample was aliquoted into a lysing matrix E tube (LMT) (**Figure 2.4**) and the weight recorded. A preservation technique using RNA-later media was considered however this does not improve DNA yield in frozen samples (Gorzelak et al., 2015). The remaining diluted stool solution was stored in two duplicate tubes and all three samples were immediately stored at -80°C until DNA extraction.

Figure 2.4 Lysing matrix tube (2ml): Each contains 1.4mm ceramic spheres, 0.1mm silica spheres, and one 4mm glass bead used for mechanical bead-beating of the sample to lyse bacterial cells for DNA extraction

2.6.2.1.2 DNA Extraction protocol

Figure 2.5 presents an overall summary of the steps of DNA extraction. The detailed protocol is as follows:

1. 122 µl mammalian tissue (MT) buffer was added to the LMT.
2. A second buffer, 600 µl sodium phosphate buffer, was added to the LMT to wash the sample.
3. The sample was homogenised in a FastPrep® instrument (MP Biomedicals, Sana Ana, CA) for 30 seconds at a speed setting of 6.5 m/s for 30 seconds.

4. The LMT was centrifuged at 14,000 x g for 10 minutes to pellet the debris.
5. The supernatant was transferred to a 1.5 ml eppendorf tube for protein removal. 250 µl protein precipitation solution was added and the sample was mixed by shaking the tube by hand 10 times.
6. The sample was centrifuged at 14000 x g for 5 minutes to pellet the precipitate and the supernatant was transferred to a clean 15 ml falcon tube.
7. 1ml of Binding Matrix Suspension was added and the tube was inverted by hand for 2 minutes to allow binding of the DNA. The tube was left on a rack for 3 minutes at room temperature to allow settling of the silica matrix.
8. A majority of the supernatant was discarded and the DNA-bound mix was resupsended in the remaining supernatant using a filter pipette. Approximately 600 µl of the mixture was centrifuged at 14,000 x g for 1 minute in a SPIN filter and catch tube system. Centrifugation allowed the DNA-bound matrix to be retained in the spin filter, while the liquid phase passed into the catch tube.
9. The catch tube was emptied and blotted on tissue. The pellet was resuspended in 500 µl salt ethanol wash solution (SEWS-M) and centrifuged at 14,000 x g for 2 minutes to wash away impurities.
10. The catch tube was emptied, replaced and the sample centrifuged a second time at 14,000 x g for 2 minutes to dry the matrix of residual wash solution.
11. The catch tube was discarded and a replaced with a new catch tube and the sample was air dried for 5 minutes under an extraction hood at room temperature.
12. The matrix was resuspended in 180 µl of DNase, pyrogen-free water (DES) using a filter pipette tip and incubated for 5 minutes at 55°C in an oven (Shake 'n' Stack Hybridisation Oven, Thermo Scientific).
13. The sample was centrifuged at 14,000 x g for 1 minute to transfer eluted DNA into the clean catch tube.
14. The sample was transferred to a sterile eppendorf and centrifuged at 14,000 x g for 1 minute.
15. DNA concentration was measured using a NanoDrop ND1000 instrument (Thermo Scientific, Waltham, MA, USA).
16. The sample was briefly vortexed and given a pulse spin. 75-90 µl of the sample was aliquoted into a second eppendorf for storage at -20°C, whilst the remaining sample was stored at 4°C for qPCR.

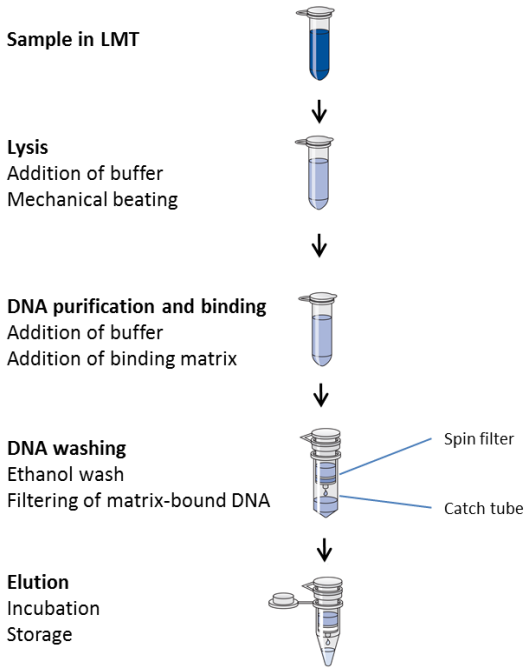

Figure 2.5 Summary of DNA extraction procedures using the FastDNA™ SPIN kit for soil. (MP Biomedicals Europe, Illkirch-Graffenstaden, France)

2.6.2.2 Rationale for choice of method

There are multiple methods for the quantification of microbiota from stool samples (**Table 2.3**). Culture dependent techniques rely on a comprehensive understanding of the nutritional and growth requirements of the microorganisms of interest, and more than 30% of the total GI microbiota are not cultivable (Fraher et al., 2012). Therefore, culture-independent techniques have revolutionised understanding of the composition and functionality of the microbiota. These methods are based on detecting variance of small subunit ribosomal RNA (16Sr RNA) sequences, which are present in all living organisms, and allows assessment of qualitative and quantitative information on the species present (Fraher et al., 2012).

qPCR is a technique used for quantification of the GI microbiota from phylum to strain level. It is a relatively rapid technique and is reported to be the most accurate culture-independent method to evaluate total microbiota concentrations in samples (Fraher et al., 2012). It is more sensitive than FISH and enables a higher throughput, but is less expensive than other methods such as next generation sequencing. Numerous steps in the technique introduces a risk of bias (Bustin et al., 2009), however this can be attenuated by reducing the number of personnel involved in the experimental methods. As such, in this RCT, HS performed all sample

Table 2.3 Summary of methods used to characterise the GI microbiota

Method	Description	Advantages	Disadvantages
Culture	Selective or differential media are used to isolate bacteria. Selective media stimulates growth of specific bacteria whereas differential media allow discrimination of closely related species	• Cheap • Quantitative or qualitative	• Labour intensive • Many species unculturable • Misses species that depend on other species for growth
PCR (polymerase chain reaction)	DNA is extracted from samples and 16S rRNA is amplified enzymatically throughout a heat cycling process that leads to DNA denaturation and synthesis	• Qualitative • Highly sensitive	• Not quantitative • Identification of unknown species not possible
qPCR (quantitative polymerase chain reaction)	As above but amplification of 16S rRNA target is measured in real time. Fluorescence is proportional to the concentration of the target, and can be quantified via comparison with a standard curve	• Quantitative • Most accurate technique for measurement of total bacteria • Highly sensitive	• Numerous steps increase potential for bias • Limited by primer design and choice • Labour intensive
Fingerprinting techniques e.g. DGGE, T-RFLP	PCR followed by band visualisation based on separation of 16S rRNA amplicons by application of electrophoretic current (DGGE) or restriction endonucleases (T-RFLP)	• Cheap • Rapid • Can be quantitative if applied with other techniques	• Semi-quantitative • Labour intensive • Bias associated with PCR
Fluorescence *in situ* hybridisation (FISH)	Fluorescently labelled oligonucleotide probes hybridise complementary 16S rRNA sequences, followed by quantification by flow cytometry or manual counting	• Quantitative • No bias associated with PCR	• Labour intensive • Identification of unknown species not possible • Relies on validated probes
DNA micro-array	Fluorescently labelled oligonucleotide probes hybridise to DNA on a solid surface and fluorescence is detected by a laser	• Cheap • Rapid	• Semi-quantitative • Poor detection of low abundance species
Next-generation sequencing	PCR amplification with fluorescent signal detection is followed by analysis of the exact nucleotide sequence of a DNA molecule which is compared to sequence databases. Includes Illumina®	• High throughput • Low abundance bacteria detected • Measures of diversity possible	• High start-up cost • Data analysis is complex and requires specialist bioinformatics experience • Bias associated with PCR

processing, preparation and qPCR experiments. Furthermore, in order to optimise the technical quality of the experiment, guidelines relating to conducting and interpreting qPCR experiments were strictly adhered to (Bustin et al., 2009).

2.6.2.3 Principles of qPCR

qPCR is a commonly applied technique for quantification of double-stranded DNA in a sample. Two oligonucleotide primers that complement the gene (the target DNA template sequence) of interest, deoxynucleotide solution (dNTP), a heat stable polymerase, magnesium ions, and a fluorescent reporter are required for the reaction. The following three stages occur during each PCR cycle: denaturation, annealing and extension.

Application of high temperature (usually 95°C) denatures the DNA, and the double-stranded DNA melts into single strands (see **Figure 2.6**). The temperature and duration of application are dependent on the template sequence and the instrument used (Kubista et al., 2006). This is followed by subsequent lowering of temperature to approximately 5°C below the primer melting temperature (typically 40-75°C) to allow the primers to anneal to the sample DNA template. Application of the correct temperature in this phase is important to optimise annealing and limit amplification of non-specific DNA fragments.

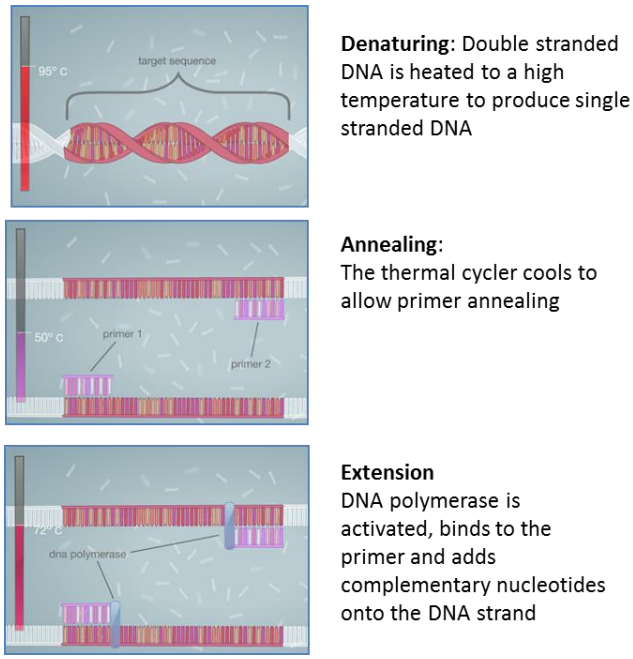

Figure 2.6 One PCR cycle involves denaturation, annealing and extension. One cycle leads to a doubling of amplicon (adapted from (Arizona State University, 2013)

The final stage involves increasing the temperature to optimise polymerase activity, which binds to the annealed primer-DNA complex, leading to synthesis of complementary DNA at a rate of up to 100 bases a second. This cycle is then repeated 40-50 times, resulting in amplification of DNA template (amplicons) of up to many millions of copies. The fluorescent reporter generates a fluorescent signal only when bound to double stranded DNA, and therefore there is a direct association between fluorescence and the number of amplicons formed (Kubista et al., 2006).

2.6.2.4 Fluorescent reporters

The process of multiple amplification cycles leading to an exponential increase in amplicon is utilised in both PCR and qPCR. However, the benefit of qPCR, or 'real-time' PCR, is the measurement of real-time amplicon accumulation according to the fluorescence generated. An effective fluorescent reporter has low background fluorescence, high fluorescence on binding to the target, and high specificity (Kubista et al., 2006). Two reporter systems are available for qPCR, SYBR green and the *Taq*Man probe (Smith and Osborn, 2009).

SYBR green is relatively cheap, but its ability to generate a fluorescent signal when bound to all double-stranded DNA is a limitation as binding to nonspecific products and primer-dimers (Section 2.6.2.5) contributes to a signal. The *Taq*Man probe avoids this problem as it derives fluorescence only when the target sequence is amplified but is more expensive. SYBR green reporter was used in this experiment, as it has routinely been used in our laboratory. The SYBR Green Supermix (Biorad, Hercules CA) contained SYBR green dye, DNA polymerase, dNTP, magnesium chloride and passive reference dyes. The passive reference dye (ROX and fluorescein) normalises well to well differences in fluorescence variation due to non-PCR related differences in fluorescence (e.g. pipetting error leading to variations in volume of the SYBR green).

2.6.2.5 Primers

qPCR primers are single-strand oligonucleotide sequences specific for the 16Sr RNA target to be amplified. Specificity of primers is important, especially when SYBR green is used as the fluorescent reporter, in order to avoid production of nonspecific PCR products that contribute to the fluorescence signal (Smith and Osborn, 2009). Primer-dimer formation occurs where primers bind to each other due to complementary bases, which interferes with the PCR

reaction. To enable practical annealing temperatures and efficient amplification, primers should be 18-24 nucleotide bases long.

Design of the qPCR experiment required initial selection of the specific primers that targeted the microbial groups or species of interest. For this RCT, a total of 12 primers were selected (see **Table 2.4**). Primers were chosen that targeted bacterial groups that have previously demonstrated to change in response to a low FODMAP intervention (e.g. Bifidobacteria, total bacteria, Clostridium cluster XIVa (Staudacher et al., 2012, Halmos et al., 2015). Others were chosen to target groups expected to increase in response to VSL#3 probiotic supplementation (Lactobacilli, *B. longum, B. adolescentis*). Finally, primers targeting some genus-level (Bacteroides, Prevotella, *Roseburia* spp.) and species-level bacteria (*F. prausnitzii, Akkermansia muciniphila, Ruminococcus bromii*) were chosen as they have been shown to alter in response to change in fibre and/or carbohydrate intake (Section 1.1.6.3.1), are considered potentially important for health and disease, or are deemed 'keystone species' (Scott et al., 2015).

In this RCT, most primers had previously been validated in our laboratory. Two primer sets had not previously been validated (*R. bromii*, *A. muciniphila*) and appropriate efficiency validation was conducted. Three primer pairs that were designed and validated by collaborators (Rowett Institute of Nutrition and Health, University of Aberdeen) performed poorly in our laboratory, with poor overall amplification (*B. breve, B. infantis*) and poor efficiency (Clostridium Cluster IV). The latter assay was repeated and continued to perform poorly, and due to insufficient time to perform further validation experiments, data from these three assays were omitted.

2.6.2.6 qPCR analysis

2.6.2.6.1 Amplification curve

DNA template in the original sample is quantified in a qPCR assay by plotting fluorescence against the cycle number. There are key aspects of the amplification cycle: baseline phase, exponential phase, cycle threshold and the linear phase (**Figure 2.7**). The baseline phase refers to the initial cycles of PCR where fluorescence is undetectable. PCR software enables conversion of baseline fluorescence of all samples to 0, also called 'normalisation'. After baseline, amplification of the template should theoretically double in each cycle, leading to an exponential increase in fluorescence, termed the exponential phase. Quantification during the assay occurs at the threshold, where the level of signal is considered significantly higher than baseline, termed the threshold cycle (C_q). This is early in the exponential phase where all the

Table 2.4 Characteristics of primers used in the RCT, including forward and reverse sequences, annealing temperature and standards used for each assay

Target	Primer name	Sequence (5'-3')	Annealing temperature C°)	Primer Reference	Standard
Universal	UniF UniR	GTGSTGCAYGGYYGTCGTCA ACGTCRTCCMCNCCTTCCTC	60	(Walker et al., 2011)	A2-183
Bacteroides spp.	g-Bfra-R-Fmod Bac708Rmod	GCTCAACCKTAAAATTGCAGTTG GCAATCGGRGTTCTTCGTG	63	(modified) (Matsuki et al., 2002) (Bartosch et al., 2004)	*B. theta* B5482
Prevotella spp.	g-Prevo-Fmod BacPreRmod	CRCRCRGTAAACGATGGATG TTGAGTTTCACCGTTGCCGG	65	(modified) (Matsuki et al., 2002) (Wood et al., 1998)	*Prevotella copri* DSM18205
Bifidobacteria	BifF g-Bifid-R	TCGCGTCYGGTGTGAAAG GGTGTTCTTCCCGATATCTACA	60	(Walker et al., 2011)	*B. adolescentis* DSM 20083
B. longum	BlonF BlonR	CAGTTGATCGCATGGTCTT TACCCGTCGAAGCCAC	60	(Malinen et al., 2005)	*B. longum* DSM 20219
B. adolescentis	Bif164F BiADO-2	GGGTGGTAATGCCGGATG CGAAGGGCTTGCTCCCAGT	60	(Ramirez-Farias et al., 2009)	*B. adolescentis* DSM 20083
Clostridium Cluster XIVa	Erec482F Erec870R	CGGTACCTGACTAAGAAGC AGTTTYATTCTTGCGAACG	60	(Ramirez-Farias et al., 2009)	*R. hominis* A2-183
Roseburia spp. & *E. rectale*	RrecF Rrec630mR	GCGGTRCGGCAAGTCTGA CCTCCGACACTCTAGTMCGAC	63	(Walker et al., 2011)	*R. hominis* A2-183
F. prausnitzii	FPR-2F Fprau645mR	GGAGGAAGAAGGTCTTCGG AATTCCGCCTACCTCTGCACT	60	(Ramirez-Farias et al., 2009)	*F. prausnitzii* A2-165
R. bromii	Rflbr730F RbromR	GGCGGCYTRCTGGGCTTT CAACTTTCCCCGAAGGGCACCTA	60	(Salonen et al., 2014)	*R. bromii* L2-63
A. muciniphila	AM1 AM2	CAGCACGTGAAGGTGGGGAC CCTTGCGGTTGGCTTCAGAT	60	(Collado et al., 2007)	DSM 22959
Lactobacilli	LAC-1 Lab 0677	AGCAGTAGGGAATCTTCCA CACCGCTACACATGGAG	60	(Malinen et al., 2005)	*L. reuteri* DSM 20016

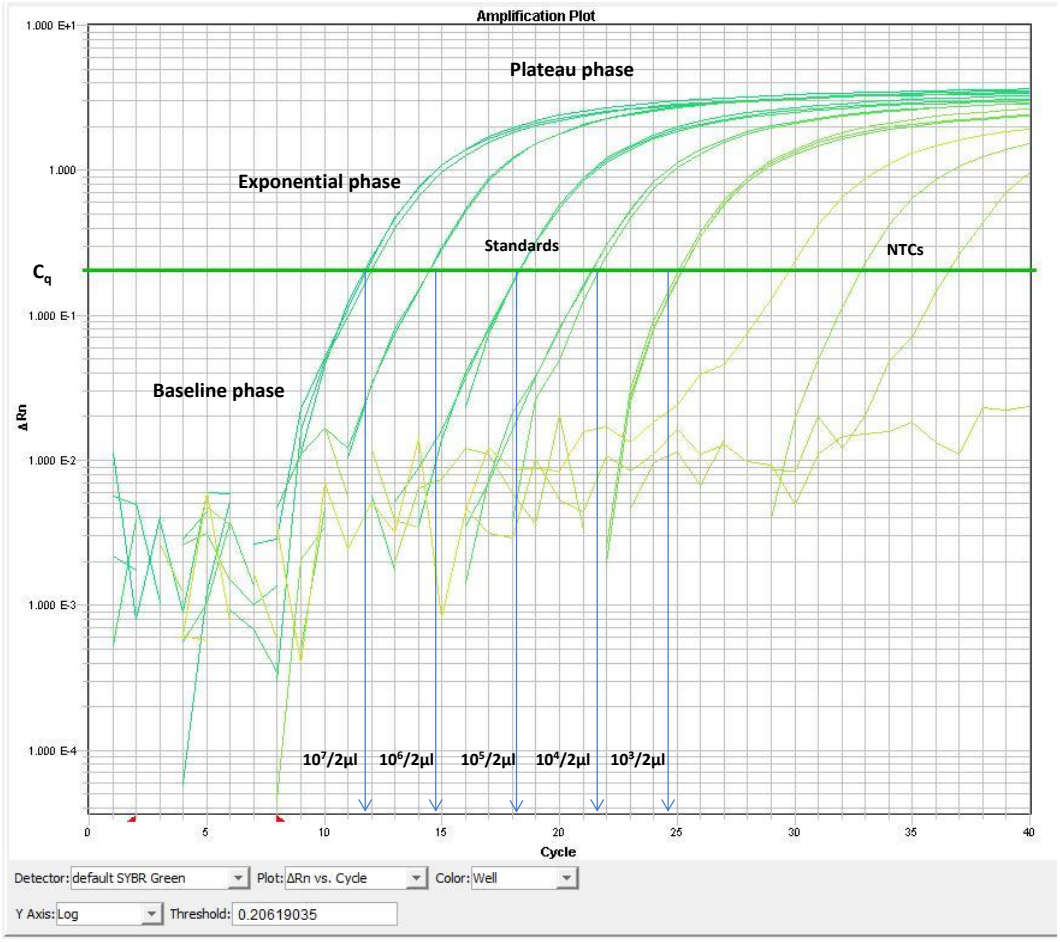

Figure 2.7 Amplification plot for Roseburia standards (*R. hominis* A2-183): The fluorescence (ΔRn) is plotted against cycle number for five dilutions of standard (10^7/2µl to 10^3/2µl) and no-template controls (NTCs). The threshold cycle (C_q) is the cycle at which quantification takes place

reagents are still in excess and amplification products will not compete with primer annealing (Smith and Osborn, 2009). There is an increase in C_q with reducing concentrations of starting template. The plateau phase occurs where the reaction is limited by insufficient polymerase, reporter or primer products or the PCR products interfere with annealing (Kubista et al., 2006)

2.6.2.6.2 Standard curve

For absolute quantification of the original DNA template, copy numbers can be determined from a standard curve (**Figure 2.8**). This curve is produced from amplification of the pure target (standards) at a range of concentrations (in triplicate) covering the expected concentrations in the sample (Taylor et al., 2010). The C_q of each standard dilution is plotted

against the known standard copies, and then abundance of the sample target in each qPCR plate well is determined by the software by comparison of the sample C_q to the standard curve (Smith and Osborn, 2009). The standard curve is also used to assess the reaction efficiency (Section 2.6.2.7.3). Using this method of quantification, the quality of the standard curve is critical for accuracy, and can be affected by accuracy of pipetting and stability of the diluted standards. In order to improve transparency of the data generated, reporting of a number of assay parameters is important according to the minimum information for publication of qPCR experiments (MIQE) (Bustin et al., 2009). Some of these are detailed in the following sections.

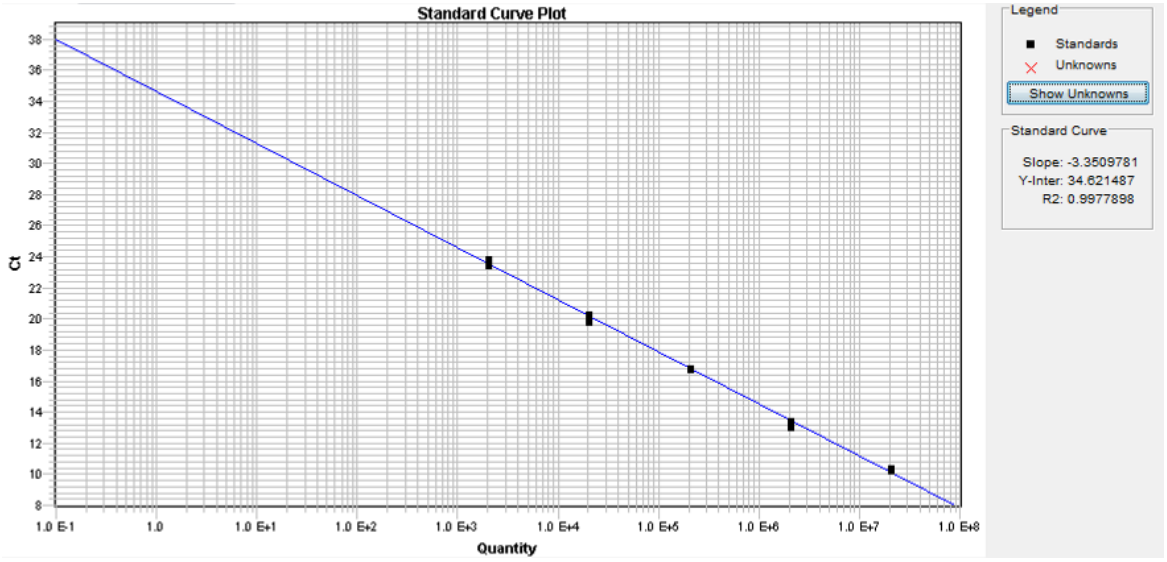

Figure 2.8 Standard curve for Prevotella assay (*P. copri* DSM 18205): A linear regression curve for C_q (termed Ct here) is plotted against known concentrations in the dilution series

2.6.2.7 qPCR validation and optimisation

2.6.2.7.1 Controls and limit of detection

All qPCR assays included two no-template controls (NTC), molecular biology grade water (MBH_20) and herring sperm DNA. These were treated identically to the samples by addition of all assay reagents except the DNA template. Fluorescence from the NTCs represented contamination or primer-dimers (Taylor et al., 2010). Sample fluorescence detected within 3.3 cycles from the NTC was considered below the detection limit (Smith and Osborn, 2009).

2.6.2.7.2 *Specificity*

Specificity of the qPCR reaction refers to the degree to which fluorescence is generated from detection of the target template rather than nonspecific products. This should be evaluated at the end of each assay by assessment of the melt curve (dissociation curve) (**Figure 2.9**). This is constructed by heating of the double-stranded template until it dissociates and loses fluorescence (Smith and Osborn, 2009). Primer-dimer products are shorter than target amplicons and will melt earlier and are easily identifiable as separate peaks (Kubista et al., 2006), whereas a sharp peak indicates amplification of one product, and good specificity of primer annealing. Where primers for genus-level bacteria were used (e.g. Bifidobacteria) a wider peak was expected due to detection of a wider range of species. A melt curve was generated and inspected for all assays. Where separate melt peaks were not clear, this was discussed with collaborators for confirmation as to whether or not this was expected for the assay.

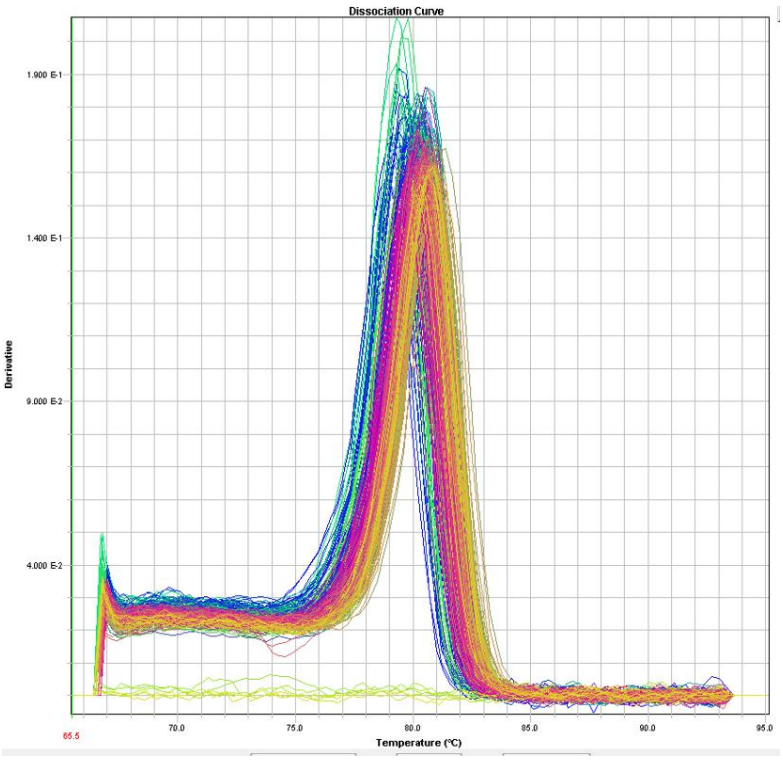

Figure 2.9 Dissociation curve for Roseburia assay for one 384-well PCR plate: The change in fluorescence is plotted against temperature.

2.6.2.7.3 *Efficiency, repeatability and sensitivity*

The efficiency of a reaction is an important indication of the success of the qPCR assay. This represents the rate at which the polymerase converts the reagents to amplicon in the assay. A

two-fold increase in amplicons per cycle represents a 100% efficient reaction. Generally, acceptable efficiency ranges between 90-110%. Low efficiency of a reaction (<90%) can be due to contamination or suboptimal annealing temperatures whereas high efficiency (>110%) is usually due to primer-dimer formation or nonspecific amplicons (Taylor et al., 2010). Efficiency is calculated based on the slope of the exponential phase of the amplification curve (Efficiency = $-1 + 10^{(-1/\text{slope})}$), whereby 100% efficiency is equivalent to a slope of -3.32. Efficiencies for the PCR experiments were calculated for this RCT and ranged between 86-102%.

All samples were analysed in triplicate to reduce the effects of technical variability. Another standard procedure to assess variability is evaluation of the correlation coefficient (r^2), or linearity, calculated from the standard curve. A low r^2 represents significant differences in C_q between replicates and a recommended minimum is 98.0 (Taylor et al., 2010). Values for r^2 across the PCR experiments for this RCT were 99.0-99.8 indicating high repeatability.

The sensitivity of a PCR reaction is a measure of the lowest number of template copies that could theoretically be detected in the assay. According to standard procedure, all samples that demonstrated fluorescence at a $C_q \leq 3.3$ cycles from the NTC C_q were considered below the detection limit due to inadequate fluorescence above background fluorescence from no-template containing wells.

2.6.2.8 qPCR protocol

Preparation for each assay was carried out in a clean area of the laboratory using filter pipette tips and molecular biology grade reagents. Samples were recoded by a researcher not involved in the study in order that HS (who was not blinded to the diet allocation) was blinded to the samples. Prior to the assays the samples were manually diluted in MBH_20 to a concentration of 25 ng/µl. Samples were then transferred to clear 2.2 ml storage plates (Greiner Bio-one) at a volume of 100 µl per well. The samples were diluted with herring sperm DNA to a final concentration of 1.25 ng/µl by an automated pipetting system (Biomek FXP Laboratory Automation Workstation, Beckman Coulter, Brea, CA, USA) into three sets of two clear 96-well skirted PCR stock plates (Greiner Bio-one), of final volume 150 µl per well and sample locations were documented. Stock plates were stored at -20°C until required. For each assay, the following protocol was followed:

1. Standard dilutions series were prepared from 10^9 copies of 16S rRNA gene standards (**Table 2.4**). Five ten-fold dilutions were prepared using 5 µg/ml herring sperm DNA starting from 10^7 copies/2 µl). For lower abundance bacteria, dilutions were prepared starting from 10^6 copies/2 µl to ensure the standard curve covered the range of expected target abundances. For the universal assay, a set of 5-fold dilutions was prepared. Standard dilutions were discarded after 1 week and prepared again where required.
2. Dried primers were diluted in MBH_20 to 100 uM as per manufacturer's instructions (Sigma-Aldrich) and stored at -20°C until required. For each assay, primers were further diluted to 10 uM in MBH_20.
3. The assay mix was prepared, by addition of SYBR green to the primers, ensuring there was sufficient for samples and NTCs in triplicate, allowing 20% extra volume for pipetting error.
4. 6 µl assay mix was pipetted into clear 384 MicroAmp Optical 384-well reaction plates (Applied Biosystems) by the automated pipetting system, and then 4 µl of each sample was added in triplicate wells and mixed by the same system (**Table 2.5**). This quantity of DNA (total DNA concentration 5 ng) is considered sufficient to amplify high and low abundance targets. Two plates were required for each assay due to the number of samples in the experiment. Five standards and two NTCs were included in triplicate on each plate. Baseline and follow up samples for each patient were allocated to the same plate to minimise technical variability.
5. Each qPCR plate was sealed using an optical adhesive cover and centrifuged (Platefuge Microplate Centrifuge, Benchmark Scientific). If the plate required storage prior to thermal cycling, it was covered with opaque foil and refrigerated at 4°C for no more than 2 hours.

Table 2.5 Reagents added to each well of qPCR 384 well plate

Reagent	Volume per well
SYBR green supermix	5 µl
Forward primer (10µM)	0.5 µl
Reverse primer (10µM)	0.5 µl
Sample (1.25ng/ µl)	4 µl
Total	**10 µl**

2.6.2.9 Reaction conditions

The thermal cycler used for all experiments was a 7900HT fast qPCR system (Applied Biosystems). Maintenance and calibration of the instrument had been performed annually. The reaction conditions were maintained as per usual practice in our laboratory (**Table 2.6**) and annealing temperatures were adjusted according to the assay (**Table 2.4**).

Table 2.6 qPCR reaction conditions

Step	Number of cycles	Temperature (°C)	Duration
1 Denaturation	1	95	3 minutes
2 Annealing and extension	40	95	5 seconds
	1	60*	30 seconds
3 Dissociation	1	95	10 seconds
	1	65 to 95	5 seconds

*Annealing temperature varied between primer pairs

2.6.2.10 Data analysis

Data were analysed using SDS 2.4.1 (Applied Biosystems). Absolute quantification of copies of amplicons per well was calculated by comparison of the software-generated C_q of the target template with the standard curve. Triplicate data were checked and where amplification reproducibility was poor (i.e. where C_q SD≥0.3) mean copies per well was calculated based on data from two wells. Copies per well were then converted to $\log_{10}$ cells/g faeces accounting for the original sample weight, dilutions carried out during processing and the DNA concentration for each sample. Relative abundance of bacterial groups was calculated relative to total bacteria abundance for that sample. Scatterplots were used to perform final crosschecks. For example, the sum of species-level abundance (*B. longum* and *B. adolescentis*) was expected to be lower than their genus-level abundance (e.g. Bifidobacteria). Samples were only unblinded once data were finalised and locked.

2.6.3 Markers of fermentation: Stool SCFA

Carbohydrates are major substrates for fermentation in the colon. Therefore dietary modification that alters the carbohydrate type and/or volume entering the colon may lead to alteration in fermentation dynamics. Major byproducts of carbohydrate fermentation are SCFA, which have important influences on intraluminal and mucosal metabolism in the GI tract. SCFA were measured in this RCT in order to evaluate whether the interventions modified colonic fermentation. Gas liquid chromatography (GLC) is a quick, relatively inexpensive and commonly used technique for measurement of SCFA. Sample preparation was performed by

HS, GLC was conducted by Robert Gray (Analytical Chemist, King's College London) and was supervised by Professor Kevin Whelan.

2.6.3.1 Rationale for choice of method

Measurement of SCFA production *in vivo* can be performed directly or indirectly. One direct method of measurement is by calculation of the difference between carbohydrate entering the colon and the colonic organic matter and SCFA, although this is invasive and technically difficult to perform (Millet et al., 2010). Another method involves measuring the difference in SCFA between portal and venous blood, although this is biased as it is unable to account for uptake of SCFA by colonocytes (Millet et al., 2010).

The indirect method of determining SCFA production is by measurement in stool. A major disadvantage of this is that absolute net SCFA stool concentration is not representative of production. This is because SCFA production occurs predominantly in the caecum and ascending colon in humans and its concentration declines as the luminal contents transit distally, due to absorption of a majority of SCFA (>95%). Another disadvantage of this method is that stool SCFA is affected by variable stool volume (Cummings and Macfarlane, 1991). However, measurement of stool SCFA is relatively simple, and may be useful in detecting changes in excretion (Topping and Clifton, 2001), assuming stool volume does not change.

GLC, high-performance liquid chromatography, ion exclusion chromatography and capillary electrophoresis are methods used to measure SCFA. GLC is quick, relatively inexpensive and is the most commonly used technique for measurement of SCFA. This method is a separation technique in which samples are injected into a column oven. Various chemical constituents of the sample pass through the column with a carrier gas. The sample molecules elute from the column with different retention times depending on their chemical properties. A detector identifies the compounds present by measurement of retention time, and quantification is possible by measurement of the area under the peaks in the output.

2.6.3.2 Sample preparation and storage

A 3 to 5 g aliquot of fresh stool was stored at -80°C for SCFA analysis.

2.6.3.3 SCFA extraction protocol

1. The sample was defrosted on ice for one hour.
2. The sample was weighed in a stomacher bag and the weight recorded.
3. SCFA extraction buffer was added to create a 1/4 dilution. For 100 ml SCFA extraction buffer, 0.1 g mercury chloride (Sigma, UK) and 1 g phosphoric acid (Merck, Germany), added to inhibit fermentation, were mixed with 4.5 mg 2,2-dimethylbutyric acid (internal standard; Sigma, UK) and made up to 100 ml with distilled water. The internal standard is not produced by the GI microbiota during fermentation and elutes from the column within the same temperature as the SCFA under investigation.
4. The double-bagged sample was homogenised in a stomacher (Steward Laboratory Blender stomacher 400) for 2 minutes.
5. The slurry was centrifuged in a 15 ml falcon tube at 5000 g (Beckman J2-HS, USA) at 4°C for at least 10 minutes.
6. Approximately 1 ml of the supernatant was filtered through a sterile 0.2 µm filter into a Micro-vial Snap Ring Vial with integrated 0.2 ml glass micro insert and stored at -20°C until analysis.

2.6.3.4 GLC protocol

GLC was carried out using a 9890A series GLC system (Agilent Technologies, US) equipped with a flame ionisation detector and a 220 µm internal diameter, 25 m fused silica capillary column with a film thickness of 0.25 µm (ID-BP21, SGE, Australia). The carrier gas was nitrogen. The initial oven temperature was 80°C, and increased by 10°C/min up to 145°C, and then 100°C/min up to 200°C to complete the elution. The injected sample volume was 0.2 µl, and 1.2% formic acid cleaning solution (Merck, Germany) was injected between samples to minimise carry over from the previous sample.

Calibration was undertaken using a blend of pure SCFA solutions at six different concentrations to produce calibration curves (area vs concentration). Concentrations of six SCFA (acetate, propionate, butyrate, valerate, isobutyrate and isovalerate) were obtained in duplicate and mean concentration calculated for each patient at each timepoint (µmol/g wet weight). Samples were reanalysed when reproducibility was suboptimal (variability >5%). Total SCFA concentration was calculated as the sum of the individual SCFA concentrations. The Agilent Chromatogram database (Agilent Technologies, US) was used to carry out data analysis.

2.6.4 Markers of fermentation: Stool pH

Stool pH was measured in this RCT to measure GI fermentation. Sample preparation and measurement of stool pH was performed by HS with some assistance from Ann-Katherine Perz and Mary-Jo Searle (Laboratory Technicians, King's College London).

2.6.4.1 Rationale for choice of method

Peak fermentation occurs in the caecum, which exhibits the lowest pH of the entire colon (Fallingborg et al., 1989). This is likely the best site for measuring changes in colonic fermentation and pH in response to dietary intervention but it is relatively inaccessible. As discussed above, most SCFA are absorbed in the colon, and measuring pH of the distal colon or stool may not accurately reflect change in pH related to altered fermentation that occurs in the proximal colon (Watson et al., 1972). Another limitation of measurement of distal colonic or stool pH is that it is also influenced by byproducts of protein metabolism (e.g. ammonia), which may then mask effects due to altered carbohydrate fermentation. Finally, stool pH decreases once stool is passed per rectum (Watson et al., 1972), and therefore pH varies depending on when it is measured.

The wireless motility capsule, an oral indigestible data transmitter, can accurately measure pH in real time throughout the entire GI tract (Farmer et al., 2013). However, this type of assessment is somewhat invasive and not widely available, whereas measuring stool pH is non-invasive, simple and inexpensive. Therefore, stool pH was measured in this RCT, along with stool SCFA to estimate alterations in fermentation. A standard solid phase probe was used to measure pH in undiluted samples at the end of the RCT due to laboratory space restrictions at the second site.

2.6.4.2 Sample preparation and storage

A 3 to 5 g aliquot of fresh stool was stored at -80°C for pH analysis.

2.6.4.3 pH protocol

1. The sample was defrosted on ice for 1 hour.
2. A solids pH probe (InLab® Solids Pro, Mettler Toledo) was calibrated in buffered pH stock solution (Sigma, UK) daily prior to use. The probe tip was immersed in the sample to 1.5 cm depth until a pH value was detected and confirmed (FE20 Benchtop pH meter, Mettler Toledo).

2.7 Methods of measurement and rationale: Nutrient intake

Dietary intake was measured in this RCT in order to evaluate energy, nutrient and FODMAP intake.

2.7.1 Rationale for choice of method: Unweighed diet record

Dietary intake can be measured using recall (e.g. food frequency questionnaire (FFQ), 24-hour recall) or prospective methods (e.g. diet records). All are limited in accuracy due to reporting error, the tendency for individuals to alter their dietary intake whilst being monitored, the systematic and random error associated with coding of diet records, and the limitations associated with food composition tables (Bingham, 1987). FFQ and dietary recall are commonly used in large scale epidemiological studies. A FFQ that quantifies nutrient and FODMAP intake has been validated, but overestimates nutrient intake (Barrett and Gibson, 2010). Conversely, the 24-hour recall technique is limited by difficulties with conceptualisation of portion sizes, risk of under-reporting, and inability to capture day-to-day variability in intake (Ma et al., 2009).

Weighed diet records are considered the gold standard for estimating dietary intake in controlled intervention trials, however this is burdensome for the patient. Unweighed diet records are less burdensome and correlate well with biological markers of food intake (e.g. 24 hour urine nitrogen and potassium) (Bingham et al., 1995). They also have excellent agreement with 16-day weighed food diaries compared with recall methods (Bingham et al., 1994) and inclusion of portion size photographs improves accuracy (Bingham et al., 1994).

Seven days of dietary intake data are required for a $\pm$ 10% precision for energy intake (Bingham, 1987). This period of time also ensures each record represents intake from both weekdays and the weekend. Therefore, a 7-day unweighed diet record was considered the most rigorous method for estimating nutrient intake in this RCT. The record developed for this RCT (Appendix 9.5) was based on the National Diet and Nutrition Survey (NDNS) diary which includes food photographs for estimating food portions. The validity of new ways of capturing dietary intake (e.g. personal digital assistant, mobile phone) has not yet been confirmed (Illner et al., 2012) and therefore conventional paper-based methods were preferred.

2.7.2 Administration

Comprehensive advice was provided to patients at the Screening Visit and Visit 1 regarding completion of the diet records. In order to improve accuracy of records the following advice was provided verbally and in written form (Appendix 9.11):

1. Record all food and drink intake regularly throughout the day whilst at home or away from home, to minimise missing data
2. Provide as much information as possible for every food, including brand and type (e.g. Hovis granary bread, medium slice)
3. Estimate portions using household measures (e.g. teaspoon, tablespoon, cup), or from the 30 food photograph codes provided, or from food packaging
4. For home cooked recipes, record details of all ingredients, individual ingredients weights (specifying dry or cooked weight) and cooking methods
5. Record as much detail as possible for meals when eaten away from home (e.g. method of cooking, details of ingredients, portion sizes)
6. Record intake of vitamin or mineral supplements

An example diet record was presented to the patient to confirm the level of detail required. Completed food diaries were reviewed in detail at the trial visits to ensure data were sufficiently detailed and complete. A minimum of three days was considered sufficient for data analysis if the record was not complete.

2.7.3 Data input

Analysis of diet records was performed by manual input of data into specialist software. A number of software programs were assessed for use in this RCT. All software packages considered comprised food and drink composition data from McCance and Widdowson's Nutrient databank (6^{th} edition) and other minor sources, and therefore choice of software was instead based on ease of operation, ability to create new foods and recipes, and ease of exporting data. Dietplan 6 P3 (Forestfield Software, Horsham, UK) was considered the best choice for the purposes of this RCT.

Food and drink data from diet records were entered into Dietplan by matching items with appropriate foods in the software database. Data were entered by dietitian coders (HS, FR, ZB), two of which were blinded (FR, ZB). HS was experienced in dietary input and developed a

dietary data protocol for the RCT, which incorporated standard portion weights for common foods. To maximize inter-rater reliability, FR and ZB were comprehensively trained according to the data input protocol, regular meetings were held to resolve queries and to agree on methods of coding, and each patient's baseline and follow up record was inputted by one coder. Where data were ambiguous or missing, standard procedures were employed to minimise variability between coders. For example, where a portion size was missing, a previously recorded portion was used, or an average was estimated using standard guidance (Food Standards Agency, 1994). Inter-rater agreement was also assessed after input of a total of 21 records (Section 2.7.5).

Where possible, foods were matched with sufficiently representative foods already existing in the database. In the case that a food did not have an appropriate match in the database, a new food was created by adapting an existing database item and adjusting the nutrient content according to food label information. Where a new food contained a high FODMAP ingredient, this was specified in the new food name (e.g. 'cheese and onion crisps' or 'apple crumble') to alert coders in subsequent FODMAP analysis (Section 2.7.6). Similarly, due to lack of composition data for alternative foods in most software databases, wheat free (e.g. wheat free bread) and lactose free products (e.g. lactose free milk) were created as new foods using available composition data from packaging. A total of 296 new foods were created.

Cold composite foods were entered as individual ingredients (e.g. ham sandwich = ham + bread + spread), and hot composite foods were entered as a new recipe unless existing composite foods in the database were considered sufficiently representative. Weight losses on cooking were taken from standard guidelines (Food Standards Agency, 2002). A total of 180 new recipes were created. New foods and recipes were added to a local database that was created and shared between coders to avoid duplication.

2.7.4 Data cleaning

Energy, macronutrient and micronutrient intake was calculated for each patient at both timepoints. Energy intakes were examined and data were checked against the original diet records for portion size errors and corrected as appropriate where energy intake fell outside the 2.5-97.5th percentile range for gender-matched combined 2008/2009 and 2011/2012 NDNS data (1058-3315 kcal for males, 769-2587 kcal for females). The complete dataset was also sorted in descending order for portion size, protein, fat, NSP, iron, vitamin C and sodium

to check for excessive intakes that might represent input error, and where required, records were examined for errors and corrected.

2.7.5 Inter-rater agreement

Inter-rater reliability is a measure of the agreement between raters on a specified measure and is important in research where multiple independent coders are involved in data interpretation and input. Inter-rater agreement was assessed for energy and fibre intake between the three coders (HS, FR, ZB). A total of 21 diet records were randomly selected for entry in triplicate (7 baseline, 7 sham and 7 low FODMAP diet records). In response to the analysis, obvious input errors were identified and corrected. Systematic errors due to differences in coding were identified, categorised and the data input protocol was updated as required.

Inter-rater agreement for dietary record coders was assessed in a number of ways. Intraclass correlations and examination of Bland Altman plots are the most common methods utilised for measuring agreement in nutrition research (Zaki et al., 2012). Both were performed as each in isolation do not provide sufficient information for assessment of inter-rater reliability (Rankin and Stokes, 1998). Sufficient clinically important range of agreement for the Bland Altman plot was decided at a mean difference ± 1.96 SD (Bland and Altman, 2007).

2.7.6 FODMAP intake analysis

Direct analysis of the FODMAP composition of foods is performed by high performance liquid chromatography (HPLC) and enzymatic kits. A comprehensive FODMAP composition database has been produced by collaborators in Monash University based on published composition data from direct FODMAP analysis (Biesiekierski et al., 2011, Muir et al., 2007, Muir et al., 2009, Yao et al., 2014) and is incorporated into Foodworks Version 7 (Xyris Software, Australia). Dietary intake data from Dietplan for this RCT was imported into Foodworks for FODMAP analysis by collaborators at Monash University, Melbourne, Australia. This process was supervised by Dr Jane Muir (Head of Translational Nutrition Science, Monash University, Melbourne, Australia). Due to food term differences between UK Dietplan software and the Australian Foodworks software, dietary intake data from diet records required extensive formatting before being suitable for import into Foodworks. The formatting was performed by HS and SC (a research dietitian) and was performed as follows:

1. Dietplan data were extracted for each patient and exported to Excel. This included the participant number, the list of foods consumed and the weight of each food consumed.
2. An aggregate list was compiled for foods and fluids consumed throughout the RCT for very patient (23674 foods and fluids).
3. All individual carbohydrate-containing foods (with names unique to Dietplan) were converted to food terms unique to the Foodworks database. For example, Dietplan term 'apples, eating, average, raw' was converted to Foodworks term 'apple, green, FODMAP'. Where a cooked food could only be matched with a raw food Foodworks term, a proportionate weight was allocated (e.g. 85% of the raw vegetable weight) to account for water gain from cooking.
4. Recipes were created for Dietplan composite foods and individual food components were labeled with Foodworks food terms.
5. Finalised food lists were then imported into Foodworks for FODMAP composition analysis.

2.8 Methods of measurement and rationale: Acceptability

Patient-reported acceptability of treatment is an important determinant of compliance to an intervention and of its feasibility in the clinical setting. Acceptability is rarely measured in dietary studies, but it can be relatively simply evaluated using semi-structured interviews or questionnaires. Semi-structured interview requires trained interviewers and are time consuming. Acceptability questionnaires assessing whole diets or supplements assess factors such as palatability, cost, ease of use, perceived health or adverse effects, and the likelihood of future application using Likert scales (Lindsay et al., 2014, Barnard et al., 2004, Young et al., 2010). There are no validated acceptability questionnaires for the low FODMAP diet or for probiotic intervention. Therefore, a short 18-item acceptability questionnaire was purpose-designed for this RCT (Appendix 9.10). It assessed various aspects of acceptability of the interventions that were considered important for future clinical application and research. Responses were scored on Likert and dichotomous scales and items included:

Dietary acceptability:

- Ease of meal preparation and eating out
- Time spent shopping and cooking
- Palatability

- Cost
- Understanding of written information

Probiotic acceptability:

- Convenience
- Perceived adverse effects
- Knowledge of the definition of a probiotic
- Future use

2.9 Statistical analysis

Data handling and statistical analysis was conducted by HS with advice from Robert Grant (Senior Lecturer in Medical Statistics, St Georges University of London) and the Statistical Consultancy Service at King's College London. Statistical analysis was conducted according to the published guidelines (ICH, 1998). Advice regarding the statistical plan and expert opinion regarding statistical analysis was provided at annual RCT meetings by a clinical trial advisor, Dr James Lindsay (Consultant Gastroenterologist, Bart's Health NHS Trust). All statistical analysis was performed using IBM SPSS Statistics for Windows Version 22.0. Unblinding of the treatments occurred after analysis of the primary endpoints.

Prior to statistical analysis, all continuous data were examined for normality using histograms. If possible, non-normally distributed data were transformed, or non-parametric analysis was conducted. For demographic data and some nutrient intake data, continuous variables were compared using dependent t-tests and independent t-tests or Wilcoxon signed-rank and Mann-Whitney U tests for non-normally distributed data. Categorical variables were compared using the Chi-squared test.

Linear regression was performed to evaluate the effect of diet and product on continuous variables (microbiota, stool SCFA and pH, IBS-SSS, GSRS symptom severity, stool output, HRQOL). Logistic regression was performed to evaluate the effect of diet and product on categorical variables (adequate relief, proportion meeting MCID for IBS-SSS, IBS-QOL). Negative binomial regression was performed to evaluate the effect of diet and product on incidence of GI symptoms (GSRS). Adjusted regression models were performed to account for differences between groups at baseline and bootstrapping of confidence intervals (95%) was

computed due to non normal data. Interaction terms were added into regression models to check for interactions between the two independent variables (diet, product).

Where more than two groups were compared (e.g. subgroup analysis), the Chi-squared test was used for categorical variables, and Kruskal-Wallis tests were used for non-normally distributed continuous data and one-way analysis of variance (ANOVA) were used for normally distributed continuous data with Tukey's post hoc tests where the assumption of homogeneity of variances was met. Welch tests with Games-Howell post hoc tests were performed where homogeneity of variance was not met. Pearson's product-moment correlation was run to assess the relationship between baseline Bifidobacteria concentration and change in Bifidobacteria concentration. Nutrient intake data were compared between diet groups using analysis of covariance (ANCOVA), adjusting for baseline intake, where assumptions of normality, independence of covariate and treatment effect and homogeneity of regression slopes were met. Proportion of patients meeting dietary reference values (DRVs) was compared within groups using the McNemar's test. Multiple logistic regression was run to evaluate predictors of clinical response.

Data are presented as summary data e.g. mean (SD) or number (%) with estimates and 95% confidence intervals (CI) and a 2-sided p value. Differences were considered significant where $p \leq 0.05$.

2.9.1 Analysis sets

The primary analysis was based on the intention to treat (ITT) dataset, which comprised all randomised patients including withdrawals and non-compliers. The ITT analysis is conservative as it avoids optimistic estimates of efficacy by accepting that deviations from the protocol and poor compliance are realistic in clinical practice (Gupta, 2011). Sensitivity analyses are important to evaluate the credibility of the primary findings (Thabane et al., 2013). Sensitivity analyses on the per protocol (PP) dataset was performed for the primary endpoints. The PP dataset was comprised of data only from the subset of patients who did not violate the protocol, were compliant with the interventions and completed the trial. This analysis set helps to estimate the efficacy of the interventions in those who received the intervention for the intended duration, but may be biased as adherence may be related to the treatment or the condition itself (ICH, 1998).

2.9.2 Missing and ambiguous data

Every effort was made to minimise missing data. Questionnaires completed at the research site were checked immediately to ensure no data points were missing. Missing data points for 7-day symptom and stool records were considered 'missing completely at random', which are less likely to bias outcomes (Sterne et al., 2009). Missing data for withdrawn patients is inevitable in RCTs. In order to perform an ITT analysis, imputation was required to create a complete ITT dataset.

There is no universally accepted method for handling missing data. Moreover, a trial is regarded as valid if the methods for dealing with missing data are 'sensible' and are defined *a priori* (ICH, 1998). Single imputation methods include imputing the last observation or imputing the mean of the observed values. Both of these methods are straightforward to implement but can lead to underestimation of SD. Alternatively, multiple imputation creates an imputed dataset by combining results from multiple created datasets using regression. However, it is computationally complex and has its own statistical pitfalls, described elsewhere (Sterne et al., 2009). It was decided *a priori* that multiple imputation would be used for missing data if there was >10% data points missing. Ignoring missing data from withdrawn patients, the missing data in this RCT were limited to the GSRS and were minimal (<2% of all data points). Therefore, it was decided that multiple imputation was not warranted and a last observation carried forward method was employed.

3 Design, development and evaluation of the sham diet

3.1 Introduction

The gold standard method for investigating the effectiveness of a drug or nutrient (e.g. supplement) intervention is the double blind, placebo-controlled RCT. This presents a problem in dietary research firstly due to the difficulty of blinding whole diets, and secondly due to the challenge of employing an appropriate placebo control. Feeding studies are a potential solution to both of these problems as the intervention diet can be created as almost indistinguishable to the control diet, and with extreme effort both the patient and the investigator can be blinded to both diets. However, feeding studies are burdensome for the participant and the researcher in terms of time and monetary costs and have limited external validity as in routine clinical practice patients are not fed a therapeutic diet in a controlled environment. In the clinical setting dietary alteration is an outcome of transmission of advice from the practitioner, which is implemented by the patient, and is influenced by numerous factors relating to food beliefs, motivation and other aspects of behaviour change. Therefore, a clinical trial where dietary advice is given to free-living patients is more representative of what is achievable in the clinical setting and therefore the results will have far greater external validity.

Three suitable options exist for control interventions in IBS dietary advice trials. The first two are healthy eating advice or dietary advice based on accepted national guidelines (NICE, 2015), and the latter has been implemented in a recent RCT (Bohn et al., 2015). However, both present difficulties for a number of reasons. Advice cannot be applied homogenously to patients due to variability in baseline dietary intake. Blinding is difficult as both strategies are widely recognised. Furthermore, neither healthy eating nor national guideline dietary advice fit the criteria of a placebo, as both are active interventions that might influence GI symptoms. The third option for a control intervention is a formulated diet that includes dietary advice to modify food intake but that does not alter nutrients or the specific food component (e.g. FODMAPs) being investigated, also known as a ‘sham diet’.

A sham diet holds a number of advantages as a placebo in IBS dietary intervention studies. It can be tailored to appear as an exclusion diet, and therefore offers potential as a convincing placebo comparison to the low FODMAP diet. It has been recommended that the number of foods removed in a sham exclusion diet be comparable to the intervention diet (Yao et al., 2013), however detailed guidance for development and implementation of sham diets in IBS and indeed other disease states is scarce.

Despite sham dietary advice being a gold standard in dietary research, there are only a limited number of studies that have used it as a placebo control for dietary advice hypothesised to improve physical symptoms. These studies included patients with IBS (Atkinson et al., 2004), anal fissure (Carroccio et al., 2013) and non-GI conditions including migraine (Mitchell et al., 2011) and bulimia nervosa (Dalvit-McPhillips, 1984). In these studies, the foods restricted in the sham group were either chosen based on prior experience of the foods being well tolerated in that patient population (Carroccio et al., 2013) or according to patients' baseline diet (Dalvit-McPhillips, 1984). Generally, little information was provided on the design of the sham diet, and no studies reported the success of blinding, which is important where outcomes are based on subjective measures.

Sham dietary advice as a placebo control comparison to low FODMAP dietary advice has never been used and would need to fulfil a number of criteria. It would need to: 1) be a convincing exclusion diet, 2) be feasible to follow, 3) restrict an equivalent number of foods compared with the low FODMAP diet, 4) modify dietary carbohydrate sources, as for ethical purposes patients have to be informed that the active intervention diet involves altering carbohydrate intake, and 5) have no impact on nutrient or FODMAP intake, particularly fibre intake, which may impact on symptoms (Moayyedi et al., 2014) and stool microbiota (David et al., 2014). There are no established sham exclusion diets that fit the requirements described above.

3.2 Aim of this chapter

The aim of this chapter is to describe the design, development and evaluation of a novel sham diet for use in a low FODMAP dietary advice RCT in patients with IBS.

The chapter is divided into 2 parts and is set out as follows:

1. Methods for the design, development and evaluation of the sham diet, which included a pilot study and an interim analysis (Section 3.3)
2. Results of the interim analysis (Section 3.4)

3.3 Methods

3.3.1 Design of the diet

The sham diet was developed by HS in conjunction with Prof Kevin Whelan and Dr Miranda Lomer. Foods to be included in the sham diet were selected using the following method:

1. Preliminary suitable and unsuitable low FODMAP diet food lists were used as a starting point for creation of suitable and unsuitable food lists for the sham diet. Food groups were addressed individually and breads and cereals were considered first as this group contributes the most to fructan intake (Dunn et al., 2011). For the sham diet, foods/products were allocated to suitable and unsuitable lists based upon the need to create some restriction, whilst neither increasing nor decreasing fructan and fibre intake. For example, wheat products were allocated to the suitable list, and cereal grains less commonly consumed in the UK (e.g. millet, rye) and products manufactured from these grains (e.g. bread, pasta, breakfast cereal) were allocated as unsuitable.
2. Fruit, vegetables and pulses contribute significantly to FODMAP intake (Muir et al., 2009, Yao et al., 2014, Biesiekierski et al., 2011), and therefore these foods required careful consideration regarding their suitability in the sham diet. High FODMAP foods regularly consumed in the UK diet (e.g. apple, pear, pulses) were allocated to the suitable list in order to maintain FODMAP intake. Foods were also assigned to the suitable list if restriction would impact intake of other high FODMAP foods. For example, tomato is often consumed in dishes that contain high FODMAP vegetables (e.g. onion and garlic) and therefore this was assigned to the suitable list. Conversely, approximately 50% of the fruits and vegetables considered suitable on the low FODMAP diet were assigned to the unsuitable list, and preference was given to those fruits and vegetables less likely to affect intake of other foods.
3. Dairy and dairy alternative products were allocated to the suitable list, to ensure lactose intake was maintained. Meat and meat alternatives and fat sources were broadly allocated to the suitable list, except for where an unsuitable food was included in a meat-containing mixed meal. For example, a meat-containing ready meal containing chives would be considered unsuitable as chives were allocated to the unsuitable list.
4. The habitual dietary intake of individuals with IBS from a previous study (Staudacher et al., 2012) was examined. The top 10% of foods consumed, by energy and carbohydrate

content and total weight, were transferred to the suitable list in order to promote maintenance of nutrient intake.

5. The number of unsuitable foods on the sham diet was confirmed as approximately equivalent to that of the low FODMAP diet.

3.3.2 Design of the dietary resource

Access to written dietary resources has been associated with greater likelihood of response to low FODMAP dietary advice (Gearry et al., 2009). Therefore, a written resource was designed for both the low FODMAP diet and sham diet groups (Appendix 9.1). The content was limited to suitable and unsuitable food lists, and pictures and other potentially persuasive sections (e.g. mechanisms of action of the low FODMAP diet) that are usually included in clinical resources were not included to maintain blinding and to minimise and equalise the placebo effect across groups. General format, length of the resource and wording on generic information (e.g. advice on caffeine, alcohol intake) was identical.

3.3.3 Development

The diet was reviewed by members of the research team (one senior academic dietitian, one non gastroenterology research/clinical dietitian and one clinical gastroenterology dietitian) and one independent researcher. There was a consensus that the diet was not difficult enough, which might increase the risk of unblinding. To address this, it was recommended that two additional staple carbohydrate foods be allocated to the unsuitable list. Based on previous dietary intake data, potato, oats and rice were the three most commonly consumed non-wheat carbohydrate sources. Therefore, to increase the difficulty of the diet, oats and rice were allocated to the unsuitable list, but potatoes were not.

3.3.4 Evaluation

After review and subsequent amendment of the sham diet, it was evaluated both in a pilot study in healthy individuals and then in an interim analysis of the RCT.

3.3.4.1 Pilot study

The pilot study aimed to assess the feasibility of the sham diet, its success as a convincing exclusion diet and its nutrient and FODMAP content. An uncontrolled blinded pilot study was performed in healthy individuals (n=7). Individuals were advised that the diet was a test diet, but were not aware of the purpose of the diet. Individuals completed a 3-day diet record at

baseline that covered a period of one weekend day and two weekdays and were advised to continue their habitual diet during this period. Following the baseline period, individuals were advised on following the sham diet, and were provided the written resource. Individuals followed the sham diet for three days whilst completing a second 3-day diet record. At the end of the pilot study, a purpose-designed acceptability questionnaire was completed to provide feedback on feasibility and credibility of the diet. This provided information on convenience, cost, degree of change to the diet, quality of the resource, and a blinding question (i.e. 'how surprised would you be if I now told you this was a "make believe" diet').

Energy and nutrient intake was analysed using Dietplan 6 P3 (Forestfield Software, Horsham, UK) and FODMAP intake was analysed by collaborators at Monash University, Melbourne, Australia using Foodworks Version 7 (Xyris Software, Australia). This process was supervised by Dr Jane Muir (Head of Translational Nutrition Science, Monash University, Melbourne, Australia). Data for FODMAP analysis was formatted by HS prior to analysis, as described in Section 2.7.6.

Demographic and dietary data are presented in Appendix 9.12. There were no differences in macronutrient or FODMAP intake between baseline and sham diet. There was higher intake of NSP during the sham diet compared with baseline (17.3 g/d vs 13.4 g/d, p=0.043). On examination of individual intakes, there was a large increase in NSP intake for four individuals on the sham diet compared with baseline (mean 6 g/d), which was due to large portions of isolated high fibre foods (e.g. All bran, legumes and nuts).

Regarding acceptability of the diet (data not shown), difficulty of meal preparation, flavour of meals, and money and time spent food shopping were no different for the sham diet compared with baseline. Finding suitable foods when eating out was a little more difficult and most individuals reported making many changes to their diet, indicating it led to substantial changes in dietary choice. When individuals were verbally questioned regarding how they would feel when told that the diet was a 'make believe' diet, most individuals expected it a little (3/7) or were neutral or surprised (2/7).

These results confirmed that the sham diet was not substantially different from baseline for energy, macronutrient and FODMAP intake, was feasible to follow, and was convincing as an exclusion diet. However, the increased NSP intake on the sham diet compared with baseline

was a concern due to its potential effect on IBS symptoms (Moayyedi et al., 2014). This may have represented a true increase in NSP intake. Conversely, the intervention also came at least one week after the baseline recording period which may have led to an order effect. Furthermore, a 7-day sham intervention may have more precisely captured true NSP intake, as 7-day diet records are preferable for measuring intake than 3-day diet records (Bingham, 1987).

A number of actions were taken to minimise changes in NSP intake for patients randomised to sham dietary advice in the RCT. Firstly, attention to fibre intake was emphasised by addition of a fibre counter to the both sham and low FODMAP resources. This would serve as a reminder to the dietitians (HS, RN) to attempt to maintain fibre intake in those allocated to sham dietary advice and to tailor advice accordingly (e.g. emphasise continuation of a low or high fibre wheat breakfast cereal, depending upon habitual dietary intake). Secondly, weekly phone calls to patients in the sham diet group were used to screen for unusually high fibre intakes. Lastly, due to the uncertainty about the effect of the sham diet on NSP intake, an interim analysis was planned after 20 patients had been recruited to the RCT.

3.3.4.2 Interim analysis

An *a priori* interim analysis of dietary intake was performed after 20 patients had been randomised to the RCT to confirm whether nutrient and FODMAP content of the sham diet was maintained compared with baseline (habitual diet). Seven-day diet records were analysed for nutrient and FODMAP content at baseline and follow up using Dietplan 6 P3 (Forestfield Software, Horsham, UK) and Foodworks Version 7 (Xyris Software, Australia), respectively. Data for FODMAP analysis was prepared as described in Section 2.7.6. The interim analysis was conducted by HS who was blinded to the dietary allocation (i.e. whether diet records for the analysis were allocated to sham diet or low FODMAP diet) throughout the data input and analysis process (ICH, 1998). In addition to analysis of dietary intake, success of blinding was measured by asking patients to guess their dietary treatment group allocation.

3.3.4.3 Statistical analysis of the interim analysis

Non parametric tests were used as data did not meet the assumptions for ANCOVA due to non-normality and inequality of variances. Wilcoxon signed-ranks tests were used to compare dietary intake within groups and Mann-Whitney U tests were used to compare dietary intake between sham diet and low FODMAP diet groups at each timepoint and for change in intake.

3.4 Results of the interim analysis

A total of 20 patients were included in the analysis (11 sham diet, 9 low FODMAP diet).

3.4.1 Nutrient intake

There was no difference in nutrient intake between sham diet and low FODMAP diet groups at baseline (**Table 3.1**). Similarly, there were no differences in energy, NSP or macronutrient intake at follow up compared with baseline within each group, except for carbohydrate and starch in the low FODMAP group, which were lower at follow up compared with baseline.

3.4.2 FODMAP intake

There were no differences in FODMAP intake at baseline between groups, except for fructans, which was lower in the sham group compared with the low FODMAP group ($p=0.028$). There were no differences in total or individual FODMAP intake in the sham group at follow up compared with baseline, however there was a lower total FODMAP, fructans, sorbitol and mannitol intake in the low FODMAP group at follow up compared with baseline ($p<0.05$), and intake of total FODMAPs at follow up was lower compared with the sham diet group ($p=0.011$).

3.4.3 Blinding

A majority of the patients in the interim analysis who received sham dietary advice guessed their allocation correctly (9/11, 82%). One patient guessed they had been allocated to the low FODMAP group and one was unsure.

Table 3.1 Energy, nutrient and FODMAP intake from food and fluid in the interim analysis of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=20)

	Sham diet (n=11)			Low FODMAP diet (n=9)			Baseline Sham vs low FODMAP	Follow up Sham vs low FODMAP	Change between baseline and follow up		
	Baseline	Follow up	p[#]	Baseline	Follow up	p[#]	p*	p*	Sham diet	Low FODMAP diet	P*
Energy (kcal/d)	1819 (424)	1772 (695)	0.782	2020 (618)	1775 (358)	0.074	0.450	0.990	-47 (521)	-245 (376)	0.257
Protein (g/d)	69 (16)	68 (23)	0.721	72 (20)	73 (15)	0.721	0.597	0.564	-1 (15)	1 (13)	0.940
Fat (g/d)	79 (19)	80 (37)	0.959	82 (24)	74 (15)	0.386	0.821	0.671	1 (31)	-8 (26)	0.545
Carbohydrate (g/d)	199 (64)	189 (74)	0.616	217 (55)	183 (56)	**0.017**	0.326	0.843	-10 (62)	-34 (32)	0.364
Starch (g/d)	99 (42)	94 (41)	0.260	125 (35)	103 (40)	**0.047**	0.151	0.603	-5 (32)	-22 (32)	0.257
Sugars (g/d)	87 (31)	87 (37)	0.959	84 (30)	74 (23)	0.203	0.821	0.338	0 (38)	-10 (26)	0.597
NSP (g/d)	10.5 (5.3)	11.2 (5.6)	0.610	13.0 (3.9)	11.9 (4.3)	0.953	0.326	0.628	0.7 (2.2)	-1.1 (3.9)	0.705
FODMAPs											
Total FODMAPs (g/d)	15.6 (8.7)	14.1 (8.1)	0.878	12.4 (4.0)	6.7 (5.3)	**0.037**	0.579	**0.011**	-1.5 (6.3)	-5.7 (5.7)	0.089
Fructans (g/d)	4.1 (1.3)	4.1 (2.0)	0.508	6.3 (2.4)	2.2 (2.2)	**0.009**	**0.028**	**0.023**	0 (1.3)	-4.1 (3.0)	**0.002**
GOS (g/d)	0.7 (0.4)	0.9 (0.5)	0.386	1.1 (0.8)	1.0 (0.8)	0.445	0.165	1.000	0.2 (0.7)	-0.1 (0.8)	0.280
Lactose (g/d)	8.7 (8.6)	6.1 (5.3)	0.333	3.3 (3.8)	2.7 (3.1)	0.878	0.131	0.059	-2.6 (6.4)	-0.6 (4.5)	0.545
Total fructose (g/d)	14.1 (6.8)	16.4 (10.1)	0.515	12.0 (5.7)	11.7 (3.3)	0.575	0406	0.364	2.3 (7.9)	-0.3 (5.2)	0.762
Excess fructose (g/d)	1.0 (0.9)	1.6 (1.4)	0.126	0.8 (0.6)	0.6 (0.4)	0.646	0.650	0.199	0.6 (0.9)	-0.2 (0.6)	0.161
Sorbitol (g/d)	0.6 (0.5)	1.3 (1.7)	0.415	0.4 (0.3)	0.1 (0.1)	**0.047**	0.705	**0.031**	0.7 (1.6)	-0.3 (0.3)	0.059
Mannitol (g/d)	0.4 (0.3)	0.2 (0.2)	0.059	0.4 (0.4)	0.2 (0.1)	**0.047**	0.821	0.384	-0.2 (0.3)	-0.2 (0.4)	0.496

Values are mean (SD) *Total FODMAPs are calculated as the sum of individual carbohydrates including excess fructose (not total fructose). [#] Wilcoxon signed-ranked test *Mann-Whitney U test

3.5 Discussion

Evaluation of this novel sham diet confirmed its suitability for use in the RCT. The interim analysis confirmed maintenance of energy, NSP, macronutrient and FODMAP intake in 11 patients following sham dietary advice compared with baseline. This is reassuring given the initial pilot study in healthy individuals suggested the sham diet increased NSP intake, which was of potential concern considering fibre can modify IBS symptoms.

Acceptability outcomes from the pilot study demonstrated the sham diet was feasible to follow but did lead to some difficulties, for example, with eating out. This suggests the sham diet achieved its role as an exclusion diet for the purposes of this RCT. However, some individuals from the pilot study reported being unsurprised when told the diet was 'make believe', calling into question whether the diet was convincing as a placebo. Furthermore, most patients in the interim analysis guessed their allocation correctly. It is plausible, however, that patients allocated to the sham diet in the RCT might guess treatment allocation based on whether they experienced symptom response, as it was clear the intervention diet aimed to reduce IBS symptoms. Therefore, it is difficult to determine whether lack of symptom response, rather than poor design of the sham diet, was the reason why some patients guessed their allocation.

There were some nutrient intake findings in the low FODMAP diet group that are of interest from the interim analysis. The reduction in carbohydrate and starch intake at follow up compared with baseline is not surprising given the substantial change to carbohydrate sources in the diet and is in keeping with previous data (Staudacher et al., 2012, Bohn et al., 2015). Furthermore, the interim analysis confirmed that low FODMAP dietary advice in the absence of verbal or written explanation of the mechanisms underlying the approach was still effective in reducing FODMAP intake. A significant reduction in intake of all individual FODMAPs was not evident, and this may have been due to the small number of patients included in the analysis, which may have also been the reason for the difference in baseline intake of fructans. However, recruitment of more patients was not feasible due to time constraints.

3.6 Conclusion

Evaluation of dietary intake and acceptability data confirmed the suitability of this novel sham diet for a dietary intervention RCT in patients with IBS. Suboptimal blinding was a potential concern as this can lead to bias in studies with subjective outcome endpoints (e.g. GI symptoms). An interim analysis of GI symptom outcomes was not performed, and therefore whether suspicion of allocation was actually related to symptom response could not be verified. Furthermore, the effect of expectation bias in this RCT may be attenuated as patients were also blinded to the second intervention (i.e. probiotic/placebo product). The preservation of energy, nutrient and FODMAP intake over time in patients receiving sham dietary advice confirms the suitability of this diet as a placebo control when investigating the effect of dietary intervention (i.e. low FODMAP diet, probiotic) on the GI microbiota.

4 The effect of low FODMAP dietary advice and probiotic supplementation on clinical outcomes in irritable bowel syndrome

4.1 Introduction

The clinical effectiveness of a low FODMAP diet has not been evaluated in a placebo-controlled dietary advice study in IBS. It has been shown to lead to adequate control of symptoms in 68% of patients with IBS when provided as dietary advice compared with 23% of those following habitual diet (Staudacher et al., 2012). Other work has suggested low FODMAP dietary advice provides no significant benefit over other standard dietary advice for IBS (Bohn et al., 2015), but its benefit over placebo dietary advice study has not been tested. In the clinical situation, the placebo effect can be harnessed for the benefit of the patient, however in research it leads to difficulty to identify interventions that hold therapeutic gain. This is especially important in IBS, where placebo effect is higher than for other GI disease (e.g. IBD), and has been reported to be 20-40% (Elsenbruch and Enck, 2015). Furthermore, placebo effect might not just influence GI symptoms but also HRQOL outcomes (Eickhoff, 2008).

There is some limited evidence for the benefit of VSL#3 probiotic on IBS symptoms. Three of four supplementation RCTs that have been conducted report response in at least one IBS symptom. These studies are limited in design and methodology (Kim et al., 2003, Kim et al., 2005, Wong et al., 2015), may be underpowered to detect differences in symptom response, and compliance in these studies is infrequently reported. Therefore the benefit of VSL#3 for symptoms of IBS requires clarification.

Abdominal pain is frequently utilised as a primary endpoint in clinical trials of drugs for IBS, however, a spectrum of lower GI symptoms including bloating, flatulence and urgency, and fatigue are endorsed by patients as being important to them (Spiegel et al., 2010a). Symptoms can range from being mild and infrequent to severe and continuous. Instruments for measuring individual GI symptoms can be global or specific and can measure dimensions of frequency and/or intensity of symptom experience (Naliboff et al., 1999).

Patient-reported outcomes such as HRQOL and acceptability are important adjunct endpoints when measuring success of a therapeutic intervention. This is especially pertinent in IBS where a biomarker for clinical symptom severity is not yet established. Measurement of HRQOL is important as patients respond differently to disease based on a number of factors (e.g. coping, social support, psychological comorbidity) (Wong and Drossman, 2010). Furthermore, it can be considered a net effect of the benefits and harms of a treatment, and useful for the planning of clinical services. The effect of low FODMAP dietary intervention on HRQOL has never been

assessed in a blinded RCT, and its effect in unblinded studies is equivocal (Pedersen et al., 2014, Harvie et al., 2013). The effect of VSL#3 on HRQOL has not been comprehensively evaluated. One study reported greater HRQOL to some but not all components of an unvalidated questionnaire (Michail and Kenche, 2011), whilst another demonstrated no difference between VSL#3 and placebo for the quality of life IBS-SSS subscore (Wong et al., 2015). Furthermore, there are no studies assessing the acceptability of VSL#3 in IBS, or assessing acceptability of the low FODMAP diet, other than in a retrospective study of patients with IBD (Gearry et al., 2009). Therefore HRQOL and acceptability of these interventions requires evaluation.

4.1.1 Aim of this chapter

The aim of this chapter is to report the results for clinical outcomes from the 2x2 factorial design RCT investigating the effect of low FODMAP dietary advice and probiotic supplementation in patients with IBS. Clinical outcomes were measured using the adequate relief question (co-primary endpoint; Section 2.5.1.1), IBS-SSS (Section 2.5.1.2), GSRS (Section 2.5.1.3), BSFS (Section 2.5.1.4), SF-36 (Section 2.5.2.1) and the IBS-QOL (Section 2.5.2.2).

The hypothesis was that there is a difference in the proportion of patients reporting adequate relief of IBS symptoms between patients following low FODMAP dietary advice for four weeks compared with patients following placebo sham dietary advice (Section 2.2).

This chapter is divided into four parts, and addresses the results as follows:

1. Patient recruitment and progress, characteristics, compliance and blinding (Section 4.2)
2. GI symptoms and stool output (Section 4.4)
3. Health-related quality of life (HRQOL) (Section 4.5)
4. Acceptability of the interventions (Section 4.6)

4.2 Patient recruitment and progress, characteristics, compliance and blinding

4.2.1 Patient recruitment and progress

Patients were screened between January 2012 and October 2014. **Figure 4.1** presents the CONSORT diagram of the trial. Of the 162 potential participants who were screened, 43 were excluded due to meeting the exclusion criteria. Of the 119 who entered the second screening period (7-day symptom and dietary recording period) five failed to meet the severity criteria, five withdrew and five were lost to follow up. A total of 104 patients were recruited to the RCT

who make up the ITT population. Of these, 95 patients finished the study and nine were withdrawn. The patients who withdrew did so due to family reasons (n=1), loss to follow up (n=1), commencing antibiotics (n=6) and major protocol violation (n=1, following an extreme alternative diet instead of the allocated sham diet). Of the 95 that completed the study, all were compliant with the diet, and eight did not meet the compliance criteria for the product (probiotic/placebo) intervention, which left 87 patients in the PP analysis. The sample size target (n=100) was achieved and the final patient completed the last study visit in December 2014.

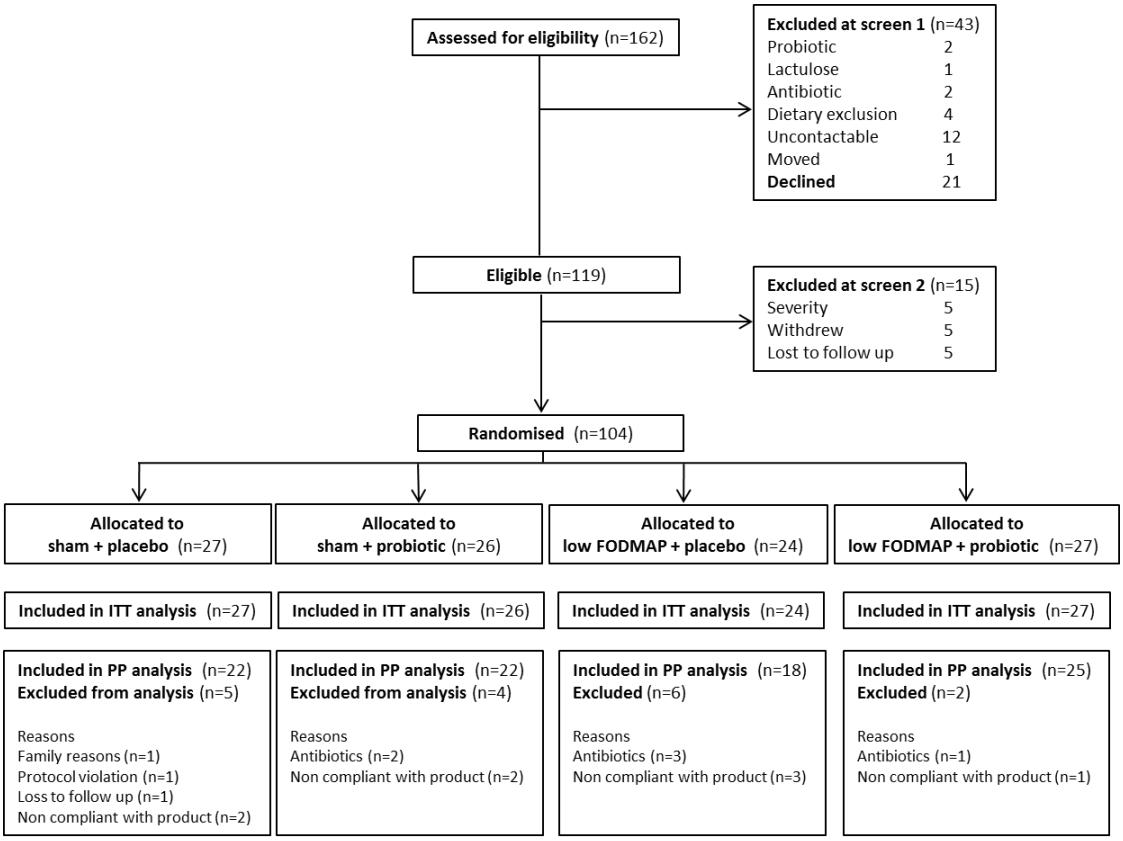

Figure 4.1 Consort diagram for the 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

4.2.2 Baseline patient demographic and clinical characteristics

Baseline characteristics are presented in **Table 4.1** There was no difference between either sham or low FODMAP diet groups or placebo and probiotic groups for any baseline variables. Most patients were white (83%) and female (67%). Most patients had IBS-D (66%) with a mean (SD) symptom duration of 6 years (8 years). Most patients did not take medications for their

symptoms. Of the 104 patients randomised, 96 were recruited from Guy's and St Thomas' NHS Foundation Trust and eight were recruited from St George's Healthcare NHS Trust.

Table 4.1 Baseline demographic and clinical characteristics for patients with IBS participating in the 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

Variable	Sham diet n=53	Low FODMAP diet n=51	p	Placebo n=51	Probiotic n=53	p
Age yrs	34 (12)	37 (12)	0.270	34 (12)	37 (12)	0.292
Female n (%)	35 (66)	35 (69)	0.836	35 (69)	35 (66)	1.000
Symptom duration mths	62 (77)	87 (111)	0.222	61 (69)	87 (115)	0.713
IBS subtype						
IBS-D n (%)	34 (64)	35 (69)		34 (67)	35 (66)	
IBS-M n (%)	12 (23)	12 (23)	0.672	10 (19)	14 (26)	0.482
IBS-U n (%)	7 (13)	4 (8)		7 (14)	4 (8)	
Current medications						
Antidiarrheal n (%)	2 (4)	3 (6)	1.000	2 (4)	3 (6)	1.000
Analgesic n (%)	0 (0)	4 (8)	0.054	3 (6)	1 (2)	0.358
Antispasmodic n (%)	6 (11)	5 (10)	1.000	8 (16)	3 (6)	0.119
Ethnicity white n (%)	44 (83)	42 (82)	1.000	41 (80)	45 (85)	0.610
Smoker n (%)	3 (6)	5 (10)	0.148	1 (2)	7 (13)	0.060
Vegetarian n (%)	1 (2)	1 (2)	1.000	0 (0)	2(4)	0.495
Weight kg	73 (19)	69 (13)	0.232	70 (14)	72 (18)	0.612
BMI kg/m^2	25 (5)	24 (4)	0.902	25 (5)	25 (5)	0.607

Values are mean (SD) unless stated

4.2.3 Compliance

Compliance to the dietary advice is reported in Section 5.2.3. Overall mean compliance to the product (probiotic or placebo) was 93% i.e. all patients took the recommended number of sachets on 93% of the days they took part in the RCT. There were eight patients (5 placebo, 3 probiotic) who were compliant fewer than 80% of days and this was not different between groups (p=0.470).

4.2.4 Blinding

The success of blinding was measured by asking patients to guess their allocation to the diet and product groups at the end of the RCT. For the dietary allocation, 34/48 (71%) of the sham diet group and 33/47 (70%) of the low FODMAP diet group guessed their allocation correctly while 10/48 (21%) of the sham diet group and 10/47 (21%) of the low FODMAP diet group were not sure of their allocation. For the product allocation, 9/45 (20%) of the placebo group

and 14/50 (28%) of the probiotic group guessed their allocation correctly and 18/45 (40%) of the placebo group and 21/50 (42%) of the probiotic group were unsure. There was no difference in responses between placebo and probiotic (p=0.514).

4.3 Results: Adverse events

Overall, the total number of adverse events reported was small. Six patients reported worsened IBS or upper GI symptoms throughout the duration of the RCT (4 sham diet, 2 low FODMAP diet; 4 placebo, 2 probiotic) and this was not different between the diet or product intervention groups (p>0.05). Other adverse events not thought to be related to the diet or product interventions were reported in 38% of patients (e.g. headache, cold, toothache) and this was not different between diet or product intervention groups (p>0.05)

4.4 Results: GI and stool output

For all outcomes, there was no interaction between the diet and product interventions and therefore the main effects for the dietary intervention and the probiotic intervention are presented individually. The primary analysis based on the ITT dataset is presented. A further analysis based on the PP population is presented for the primary outcome.

4.4.1 Adequate relief

Logistic regression was performed to assess the effect of diet and product in predicting the likelihood of adequate relief of IBS symptoms. The logistic regression model was statistically significant $\chi(3)=8.979$ (p=0.030). The model explained 11% of the variance in adequate relief and correctly classified 63% of cases. Sensitivity was 43%, specificity was 80%, positive predictive value was 62% and negative predictive value was 61%. Of the predictor variables after adjusting for baseline differences (model 2), probiotic was statistically significant (**Table 4.2**). At follow up, patients in the probiotic group had a 2.41 (95% CI 1.06, 5.52; p=0.037) greater odds of reporting adequate relief of symptoms compared with placebo. This difference was no longer evident for the analysis of the PP population (OR 1.96, 95% CI 0.81, 4.74; p=0.136). There was a trend for an effect of low FODMAP diet, with patients having a 2.18 (95% CI 0.98, 4.89; p=0.058) greater odds of reporting adequate relief compared with sham diet, and the findings were similar for the PP population (OR 2.37, 95% CI 0.98, 5.69; p=0.054). There was also no difference when intervention combinations were compared (**Table 4.3**).

Table 4.2 Adequate relief at follow up for patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

Adequate relief n (%)		Model 1		Model 2	
		OR (95% CI)	p	OR (95% CI)	p
Intention to treat analysis, n=104					
Sham diet 20/53 (38)	low FODMAP diet 29/51 (57)	2.17 (0.98, 4.83)	0.057	2.18 (0.98, 4.89)	0.058
Placebo 19/51 (37)	Probiotic 30/53 (57)	2.19 (0.99, 4.88)	0.054	2.41 (1.06, 5.52)	**0.037**
Per protocol analysis, n=87					
Sham diet 17/44 (39)	low FODMAP diet 26/43 (61)	2.36 (0.98, 5.64)	0.054	2.37 (0.98, 5.69)	0.054
Placebo 16/40 (39)	Probiotic 27/47 (64)	1.95 (0.81, 4.67)	0.136	1.96 (0.81, 4.74)	0.136

OR odds ratio; CI confidence interval; Model 1 Crude analysis; Model 2 Baseline adjusted

Table 4.3 Adequate relief for the intervention combinations at follow up for patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Intention to treat n=104		Per protocol n=87	
	AR n (%)	p[#]	AR n (%)	p[#]
Sham diet + placebo	7/27 (26)	0.051	6/22 (27)	0.081
Sham diet + probiotic	13/26 (50)		11/22 (50)	
low FODMAP diet + placebo	12/24 (50)		10/18 (56)	
low FODMAP diet + probiotic	17/27 (63)		16/25 (64)	

AR, adequate relief; [#]Chi-squared test

4.4.2 IBS-SSS

Table 4.4 presents the IBS-SSS outcomes at follow up for the ITT population after adjusting for baseline. Bootstrapped CI were computed due to non-normal data. A linear regression established that a low FODMAP diet could statistically significantly predict total IBS-SSS scores at follow up, $F(3,83)=21.34$ ($p<0.001$). The low FODMAP diet accounted for 42% of the explained variability in the total IBS-SSS score. **Figure 4.2** presents a comparison of baseline and follow up scores for total IBS-SSS score for the two dietary interventions. The low FODMAP diet could also predict all IBS-SSS subscores except for pain severity. When the PP population was evaluated, the low FODMAP diet was also able to predict the days of pain subscore (data not shown, p=0.034).

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Table 4.4 IBS-SSS at follow up for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Diet				Product			
	Sham diet (n=53)	**Low FODMAP diet (n=51)**	**Mean difference (95% CI)**	**p**	**Placebo (n=51)**	**Probiotic (n=53)**	**Mean difference (95% CI)**	**p**
IBS-SSS total (pts)	224 (89)	173 (95)	-66.3 (-94.0, -34.4)	**0.001**	207 (98)	192 (93)	-4.8 (-34.3, 24.4)	0.721
Pain severity	40 (23)	33 (24)	-8.3 (-17.5, 0.04)	0.062	38 (24)	35 (24)	-0.7 (-9.1, 8.1)	0.892
Days of pain (days)	44 (29)	30 (27)	-16.2 (-24.6, -8.0)	**0.001**	39 (28)	35 (30)	-1.7 (-10.2, 7.6)	0.690
Distension severity	40 (24)	29 (25)	-14.3 (-22.2, -6.3)	**0.002**	34 (24)	35 (26)	1.1 (-7.2, 9.6)	0.766
Satisfaction with bowels	53 (17)	42 (23)	-13.8 (-20.8, -7.0)	**0.002**	49 (22)	46 (20)	-2.8 (-10.2, 4.0)	0.459
Affecting life	47 (21)	40 (20)	-8.8 (-16.1, -1.7)	**0.022**	46 (21)	41 (20)	-3.3 (-9.8, 3.4)	0.322
Change in IBS-SSS (pts)	-44 (72)	-117 (86)	-66.3 (97.4, -35.0)	**0.001**	-78 (96)	-82 (78)	-4.8 (-34.3, 28.0)	0.750

All units are (mm) unless stated. Values are raw mean (SD) with estimated mean difference and 95% confidence interval

Table 4.5 Outcomes for patients achieving the minimal clinical important difference in IBS-SSS score for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Diet				Product			
	Sham diet (n=53)	**Low FODMAP diet (n=51)**	**OR (95% CI)**	**p**	**Placebo (n=51)**	**Probiotic (n=53)**	**OR (95% CI)**	**p**
Achieving MCID n (%)	22 (42)	37 (73)	3.42 (1.45, 8.07)	**0.005**	27 (53)	32 (60)	1.49 (0.63, 3.52)	0.363

MCID, minimal clinically important difference; OR; odds ratio; CI, confidence interval

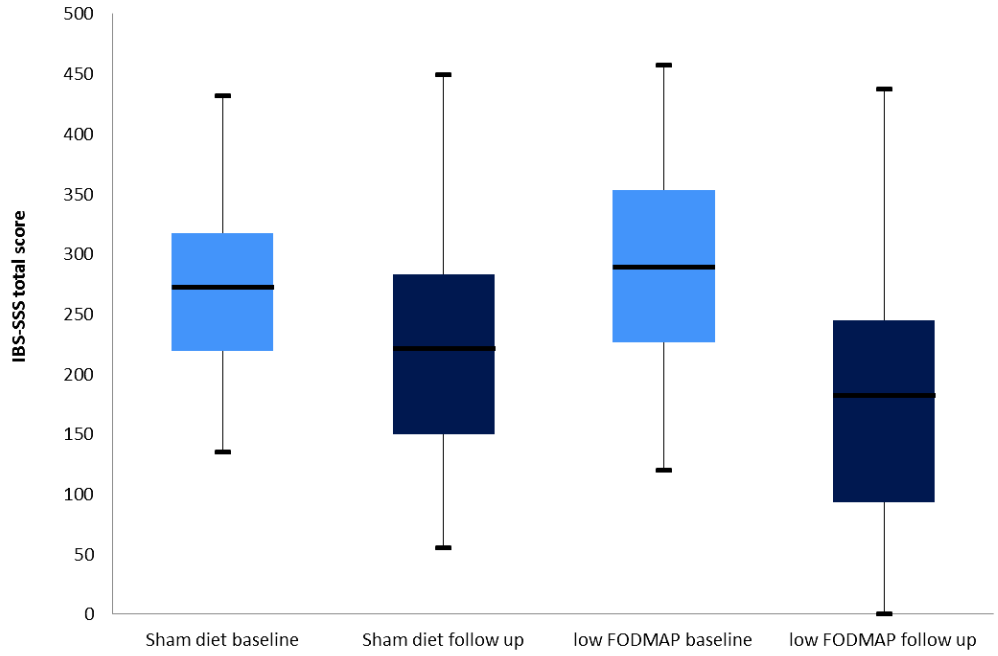


Figure 4.2 Comparison of total IBS-SSS total scores between the dietary interventions for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

There was no effect of product on total IBS-SSS score, any subscores, or change in IBS-SSS score. When examining the proportion of patients meeting the MCID for the IBS-SSS (50 point reduction in total IBS-SSS score), the logistic regression model was statistically significant $\chi(3)=19.286$ ($p<0.001$) after adjusting for baseline differences. The model explained 23% of the variance in MCID and correctly classified 69% of cases. Sensitivity was 76%, specificity was 60%, positive predictive value was 71% and negative predictive value was 66%. Patients in the low FODMAP diet group had a 3.42 (95% CI 1.45, 8.07; p=0.005) greater odds of achieving the MCID compared with the sham group, whilst there was no significant effect of the probiotic compared with placebo (OR 1.49, 95% CI 0.63, 3.52; p=0.363) (**Table 4.5**). Mean IBS-SSS scores at baseline and follow up for the diet and product interventions are presented in Appendix 9.13.

When intervention combination groups were compared, the change in IBS-SSS score was higher for the low FODMAP groups compared with the sham groups ($p<0.001$), and there was a difference between groups for the proportion meeting the MCID (sham diet + placebo 37% vs sham diet + probiotic 46% vs low FODMAP diet + placebo 71% vs low FODMAP diet +

probiotic 74%; p=0.013). Comparisons between intervention combination groups for IBS-SSS outcomes is presented in Appendix 9.14

4.4.3 GSRS

Incidence and severity of 15 symptoms and overall symptoms at follow up after adjusting for baseline values for the ITT population are presented in **Table 4.6** and **Table 4.7**. The incidence (number of days symptoms present over 7 days) of borborygmi (p=0.005), bloating (p=0.011), flatulence (p=0.023), urgency (p=0.010) and sensation of incomplete evacuation (p=0.003) were lower for the low FODMAP diet group compared with the sham diet group. When the PP population was evaluated these differences were still evident, as well as a lower incidence of overall symptoms for low FODMAP diet compared with sham diet (1.6 vs 2.3 days; p=0.028). There was no difference in incidence of individual symptoms or overall symptoms for probiotic compared with placebo.

Bootstrapped confidence intervals were computed for GSRS severity outcomes due to non-normal data. There was a lower severity of abdominal pain (p=0.010), borborygmi (p=0.003), bloating (p=0.001), belching (p=0.031), flatulence (p=0.001), urgency (p=0.001), sensation of incomplete evacuation (p=0.039) and overall symptoms (p=0.020) for the low FODMAP group compared with the sham diet group and a lower severity of flatulence for the probiotic group compared with placebo (p=0.033). When the PP population was evaluated (data not shown) these differences were still evident, and there was also a lower severity of tiredness for the low FODMAP diet group compared with the sham diet group (1.3 vs 1.5, p=0.018). GSRS incidence and severity values for baseline and follow up for the diet and product interventions are presented in Appendix 9.15.

4.4.4 Stool output

Outcomes for stool consistency, stool frequency and the proportion of stools of normal consistency (Types 3,4,5 on the BSFS) at follow up after adjusting for baseline differences for the ITT population are presented in **Table 4.8**. Bootstrapped confidence intervals were computed due to non-normal data. A linear regression established that low FODMAP dietary advice could statistically significantly predict stool consistency at follow up, $F(3,100)=11.364$ ($p<0.001$). The low FODMAP diet accounted for 23% of the explained variability in stool consistency. **Figure 4.3** presents mean stool consistency for the two dietary interventions at

Table 4.6 Gastrointestinal Symptom Rating Scale symptom incidence at follow up for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Diet				Product			
	Sham diet n=53	**Low FODMAP diet n=51**	**Exp (β) (95% CI)**	**p**	**Placebo n=51**	**Probiotic n=53**	**Exp (β) (95% CI)**	**p**
Abdominal pain	2.1 (2.0)	1.5 (1.9)	0.67 (0.40, 1.11)	0.119	1.9 (2.1)	1.7 (2.0)	0.98 (0.59, 1.61)	0.943
Heartburn	0.2 (0.6)	0.4 (1.1)	1.25 (0.52, 3.03)	0.607	0.2 (0.6)	0.4 (1.1)	1.59 (0.66, 3.85)	0.302
Acid reflux	0.3 (0.7)	0.4 (0.9)	1.47 (0.63, 3.45)	0.677	0.3 (0.6)	0.4 (0.9)	2.08 (0.85, 5.00)	0.107
Nausea	0.6 (1.4)	0.3 (0.9)	0.42 (0.18, 1.01)	0.054	0.6 (1.6)	0.3 (0.6)	1.03 (0.43, 2.44)	0.950
Borborygmi	1.9 (2.2)	1.0 (1.9)	0.43 (0.24, 0.77)	**0.005**	1.6 (2.2)	1.4 (2.1)	1.32 (0.74, 2.38)	0.350
Bloating	2.2 (2.3)	1.5 (2.0)	0.50 (0.29, 0.85)	**0.011**	1.9 (2.2)	1.8 (2.2)	0.85 (0.50, 1.43)	0.545
Belching	1.9 (1.8)	0.6 (1.4)	0.68 (0.36, 1.27)	0.226	0.9 (1.8)	0.8 (1.6)	0.67 (0.35, 1.27)	0.180
Flatulence	2.7 (2.4)	1.5 (2.0)	0.56 (0.33, 0.93)	**0.023**	2.6 (2.4)	1.7 (2.0)	0.73 (0.44, 1.20)	0.220
Constipation	0.3 (0.7)	0.4 (1.2)	1.37 (0.60, 3.13)	0.459	0.3 (1.0)	0.4 (0.9)	1.22 (0.53, 2.78)	0.644
Diarrhoea	0.5 (1.2)	0.5 (1.5)	0.79 (0.36, 1.75)	0.562	0.7 (1.5)	0.4 (1.1)	0.69 (0.32, 1.54)	0.367
Loose stool	1.3 (1.8)	1.1 (1.9)	1.00 (0.57, 1.75)	0.999	1.4 (2.1)	1.1 (1.6)	0.94 (0.54, 1.67)	0.843
Hard stool	0.2 (0.6)	0.3 (0.7)	1.15 (0.46, 2.86)	0.769	0.1 (0.4)	0.4 (0.8)	2.70 (0.97, 2.27)	0.057
Urgency	1.6 (1.8)	1.2 (1.9)	0.47 (0.26, 0.83)	**0.010**	1.5(2.0)	1.4 (1.8)	1.32 (0.76, 2.27)	0.325
Incomplete evacuation	1.7 (2.2)	0.7 (1.4)	0.41 (0.23, 0.74)	**0.003**	1.3 (2.1)	1.2 (1.8)	1.04 (0.59, 1.85)	0.877
Tiredness	2.8 (2.4)	2.0 (2.5)	0.72 (0.44, 1.16)	0.181	2.6 (2.6)	2.2 (2.3)	0.83 (0.51, 1.33)	0.431
Overall symptoms	2.3 (2.5)	1.6 (1.9)	0.66 (0.40, 1.10)	0.109	2.3 (2.5)	1.7 (2.0)	0.68 (0.41, 1.12)	0.127

Values are mean (SD) of the number of days on which the symptoms were present in seven days; Exp (β) rate ratio and 95% confidence intervals

Table 4.7 Gastrointestinal Symptom Rating Scale symptom severity at follow up for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Diet				Product			
	Sham diet (n=53)	**Low FODMAP (n=51)**	**Mean difference (95% CI)**	**p**	**Placebo (n=51)**	**Probiotic (n=53)**	**Mean difference (95% CI)**	**p**
Abdominal pain	1.1 (0.6)	0.9 (0.7)	-0.26 (-0.43, -0.06)	**0.010**	1.0 (0.7)	0.9 (0.6)	-0.03 (-0.24, 0.17)	0.753
Heartburn	0.2 (0.3)	0.2 (0.5)	0.01 (-0.10, 0.13)	0.872	0.2 (0.3)	0.2 (0.5)	0.10 (-0.02, 0.21)	0.128
Acid reflux	0.2 (0.5)	0.2 (0.4)	0.03 (-0.06, 0.13)	0.515	0.2 (0.3)	0.2 (0.4)	0.05 (-0.04, 0.14)	0.276
Nausea	0.3 (0.5)	0.3 (0.4)	-0.04 (-0.16, 0.09)	0.535	0.3 (0.6)	0.2 (0.3)	-0.09 (-0.23, 0.04)	0.191
Borborygmi	1.0 (0.7)	0.7 (0.6)	-0.34 (-0.51,-0.15)	**0.003**	0.9 (0.7)	0.8 (0.7)	0.01 (-0.17, 0.18)	0.913
Bloating	1.1 (0.7)	0.8 (0.7)	-0.35 (-0.52, -0.17)	**0.001**	1.0 (0.7)	1.0 (0.7)	-0.03 (-0.20, 0.15)	0.780
Belching	0.6 (0.7)	0.5 (0.6)	-0.18 (-0.33, -0.02)	**0.031**	0.6 (0.7)	0.5 (0.6)	-0.12 (-0.29, 0.05)	0.084
Flatulence	1.3 (0.7)	0.9 (0.6)	-0.36 (-0.55, -0.17)	**0.001**	1.2 (0.7)	1.0 (0.6)	-0.20 (-0.37, -0.01)	**0.033**
Constipation	0.3 (0.4)	0.2 (0.4)	0.05 (-0.10, 0.18)	0.559	0.2 (0.4)	0.3 (0.4)	0.06 (-0.08 , 0.19)	0.452
Diarrhoea	0.3 (0.5)	0.2 (0.5)	-0.07 (-0.20, 0.05)	0.257	0.3 (0.5)	0.2 (0.4)	-0.04 (-0.16, 0.08)	0.505
Loose stool	0.7 (0.7)	0.5 (0.6)	-0.18 (-0.37, 0.02)	0.080	0.7 (0.7)	0.5 (0.6)	-0.06 (-0.25, 0.12)	0.542
Hard stool	0.2 (0.2)	0.2 (0.3)	0.18 (-0.03, 0.13)	0.166	0.2 (0.2)	0.2 (0.3)	0.07 (-0.04, 0.16)	0.203
Urgency	0.7 (0.6)	0.6 (0.7)	-0.35 (-0.53, -0.16)	**0.001**	0.7 (0.7)	0.6 (0.6)	0.05 (-0.13, 0.24)	0.610
Incomplete evacuation	0.7 (0.7)	0.5 (0.6)	-0.21(-0.40, -0.02)	**0.039**	0.7 (0.7)	0.6 (0.6)	-0.04 (-0.22, 0.15)	0.674
Tiredness	1.3 (0.7)	1.0 (0.8)	-0.22 (-0.45, 0.01)	0.067	1.2 (0.8)	1.0 (0.8)	-0.09 (-0.32, 0.11)	0.393
Overall symptoms	1.2 (0.6)	1.0 (0.6)	-0.22 (-0.39, -0.04)	**0.020**	1.2 (0.6)	1.0 (0.6)	-0.17 (-0.34, 0.01)	0.066

Values are mean (SD) severity rated daily over seven days on a scale of 0 (absent), 1 (mild), 2 (moderate), 3 (severe), estimated mean differences and 95% confidence intervals

Table 4.8 Stool output at follow up for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Diet				Product			
	Sham diet (n=53)	**Low FODMAP diet (n=51)**	**Mean difference (95% CI)**	**p**	**Placebo (n=51)**	**Probiotic (n=53)**	**Mean difference (95% CI)**	**p**
Stool consistency	4.3 (1.1)	3.9 (1.0)	-0.50 (-0.83, -0.15)	**0.008**	4.2 (1.0)	4.0 (1.1)	-0.11 (-0.44, 0.22)	0.544
Stool frequency	12.9 (7.4)	14.0 (8.5)	-0.18 (-1.96, 1.69)	0.843	13.8 (8.3)	13.1 (7.6)	-1.41 (-3.18, 0.40)	0.136
Stool normal consistency (%)	61 (30)	67 (26)	6.23 (-3.62, 15.57)	0.200	64 (30)	64 (26)	-1.91 (-11.19, 7.54)	0.689

Values are mean (SD) with estimated mean differences and 95% confidence intervals
Stool consistency, mean Bristol Stool Form Scale type over the 7-day period;
Stool frequency, mean number of stools over the 7-day period;
Stools normal consistency, proportion of stools of types 3-5 over the 7-day period

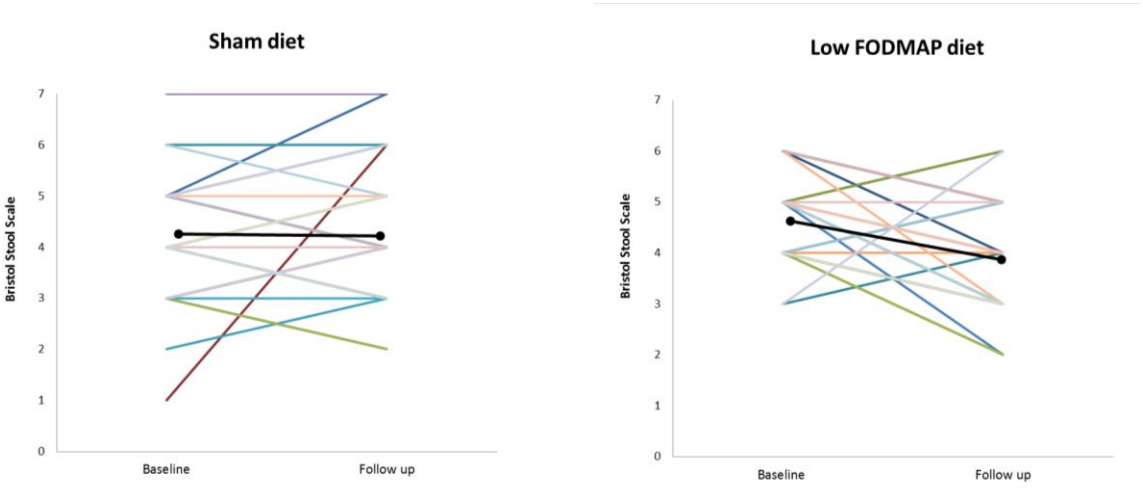


Figure 4.3 Mean stool consistency (black line) at baseline and follow up for patients with IBS in the sham diet group (n=53) and the low FODMAP diet group (n=51) participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

baseline and follow up, as well as raw data for each individual, to show variations depending upon tendency towards hard or loose stools.

There was no effect of the low FODMAP diet on stool frequency or proportion of normal stools, or for probiotic on any stool outcomes. Mean stool output data for baseline and follow up for the diet and product interventions are presented in Appendix 9.16. There were no differences for stool outcomes between diet or product interventions when the PP dataset were analysed. Neither were there any differences for stool outcomes between intervention combinations (see Appendix 9.17).

4.5 Results: HRQOL

4.5.1 Generic HRQOL

Quality of life scores for the 8 scales of the SF-36 are presented in **Table 4.9**. Bootstrapped confidence intervals were computed due to non-normal data and outcomes were adjusted for baseline. There was an effect of the low FODMAP diet on SF-36 scores. A linear regression established that the low FODMAP diet could predict two SF-36 subscales at follow up. 'Role limitations due to physical health' was significantly predicted by the low FODMAP diet $F(3,100)=25.001$ ($p<0.001$), accounting for 41% of the explained variability. 'Energy/fatigue' scale was also significantly predicted by the low FODMAP diet $F(3,100)=41.238$ ($p<0.001$),

Table 4.9 HRQOL at follow up for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Diet				Product			
	Sham diet (n=53)	**Low FODMAP diet (n=51)**	**Mean difference (95% CI)**	**p**	**Placebo (n=51)**	**Probiotic (n=53)**	**Mean difference (95% CI)**	**p**
SF-36								
Physical functioning	87.3 (22.3)	86.3 (21.3)	2.5 (-2.2, 7.7)	0.357	88.9 (19.2)	84.7 (23.8)	-3.0 (-8.6, 1.6)	0.278
Role limitations due to physical health	55.2 (39.6)	70.6 (39.3)	13.0 (1.6, 24.1)	**0.033**	62.8 (39.8)	62.7 (40.6)	-5.8 (-17.4, 7.0)	0.330
Role limitations due to emotional problems	65.4 (37.5)	64.1 (43.1)	-3.4 (-15.9, 8.5)	0.598	71.2 (38.3)	58.5 (41.3)	-6.8 (-20.2, 6.4)	0.330
Energy/fatigue	42.6 (19.9)	52.1 (23.3)	7.5 (1.6, 13.3)	**0.016**	43.9 (19.7)	50.4 (23.8)	2.3 (-3.6, 8.1)	0.427
Emotional wellbeing	63.3 (17.3)	68.7 (17.8)	4.6 (-0.2, 10.0)	0.082	66.0 (17.8)	65.8 (17.7)	0.04 (-5.1, 4.9)	0.991
Social functioning	77.8 (20.7)	73.3 (27.3)	-3.3 (-10.4, 4.2)	0.398	76.0 (25.0)	75.2 (23.6)	-1.3 (-8.7, 6.1)	0.731
Pain	65.1 (20.4)	63.5 (27.0)	3.2 (-3.5, 9.8)	0.349	61.0 (23.7)	67.5 (23.6)	2.6 (-4.8, 10.0)	0.460
General Health	56.2 (19.7)	57.5 (22.4)	3.1 (-0.9, 7.1)	0.141	55.8 (20.7)	57.8 (21.4)	2.6 (-1.9, 7.0)	0.235
IBS-QOL								
Overall	70.6 (18.1)	72.4 (19.7)	4.8 (0.02, 9.5)	0.057	68.6 (20.7)	74.3 (16.6)	0.5 (-4.0, 6.0)	0.849
Dysphoria	72.2 (20.5)	71.9 (24.7)	1.7 (-5.0, 8.2)	0.640	69.6 (24.7)	74.4 (20.3)	0.3 (-5.9, 7.4)	0.937
Interference with activity	71.2 (20.6)	72.9 (24.2)	4.9 (-1.4, 10.9)	0.120	68.9 (23.3)	75.0 (21.1)	1.4 (-4.1, 7.0)	0.640
Body Image	64.2 (22.7)	73.2 (22.7)	11.0 (5.5, 16.5)	**0.001**	64.8 (24.2)	72.2 (21.5)	-0.5 (-6.1, 4.2)	0.847
Healthy worry	71.1 (20.8)	73.0 (20.0)	2.5 (-3.7, 8.2)	0.383	69.6 (23.3)	74.4 (17.0)	2.8 (-2.7, 8.5)	0.336
Food avoidance	57.9 (29.2)	51.1 (26.7)	-0.9 (-9.2, 6.6)	0.823	53.6 (28.8)	55.5 (27.5)	-1.5 (-9.1, 5.8)	0.683
Social reaction	71.7 (22.2)	77.5 (22.4)	7.4 (1.2, 13.7)	**0.026**	71.2 (23.3)	77.7 (21.1)	1.0 (-5.8, 7.6)	0.769
Sexual	76.2 (28.6)	79.7 (24.7)	4.7 (-1.6, 11.2)	0.163	73.3 (29.3)	82.3 (23.4)	5.2 (-1.2, 12.7)	0.150
Relationships	80.5 (19.9)	81.2 (18.8)	5.4 (0.6, 10.6)	**0.041**	77.5 (20.1)	84.1 (18.1)	2.0 (-3.0, 6.8)	0.451

Values are mean (SD), estimated mean differences and 95% confidence intervals

accounting for 54% of the explained variability. There were no differences in outcomes when the PP population were analysed, however, there was also an effect of the low FODMAP diet on increasing the 'general health' scale score compared with sham diet (p=0.05). Mean baseline and follow up SF-36 scores for the diet and product intervention groups are presented in Appendix 9.18. There was no effect for probiotic on any SF-36 scales. There were no differences between intervention combinations for SF-36 scales (Appendix 9.19).

4.5.2 IBS-specific HRQOL

Quality of life scores for the 8 subscales and total score for the IBS-QOL are presented in **Table 4.9.** Bootstrapped confidence intervals were computed due to non-normal data and outcomes were adjusted for baseline. A linear regression established that the low FODMAP diet could predict three IBS-QOL subscales at follow up. The low FODMAP diet significantly predicted 'body image' ($F(3,100)=46.990$, $p<0.001$, accounting for 59% of the explained variability); 'social reaction' ($F(3,100)=32.915$, $p<0.001$, accounting for 50% of the explained variability) and 'relationships' ($F(3,100)=36.919$, $p<0.001$, accounting for 53% of the explained variability).

When the PP population was examined, there was no difference in outcomes compared with the ITT dataset, however there was an additional effect of the low FODMAP diet on increasing overall score compared with sham diet (p=0.036). Mean baseline and follow up IBS-QOL scores for the diet and product intervention groups are presented in Appendix 9.18. There was no difference in IBS-QOL scores between combination intervention groups (Appendix 9.19). Probiotic did not show any effect compared with placebo, in either the ITT or PP analysis.

Table 4.10 presents the outcomes for patients that achieved the MCID for IBS-QOL (increase of 14 points) after adjusting for baseline. Logistic regression was performed to assess the effect of diet and product in predicting the likelihood of achieving the MCID for the IBS-QOL. The logistic regression model was statistically significant $\chi(3)=22.755$ ($p<0.001$), explaining 27% of the variance and correctly classifying 66% of patients. Sensitivity was 45%, specificity was 80%. The positive predictive value was 58% and the negative predictive value was 75%. The low FODMAP diet was a significant predictor variable, with patients receiving low FODMAP dietary advice having a 2.81 (95% CI 1.15, 6.88, p=0.023) greater odds of achieving the MCID compared with those receiving sham dietary advice. Probiotic did not show any effect compared with placebo. There was no difference between groups when intervention combinations were compared (Appendix 9.19).

Table 4.10 Outcomes for patients achieving the minimal clinically important difference in IBS-QOL score for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Diet				Product			
	Sham diet (n=53)	Low FODMAP diet (n=51)	OR (95% CI)	p	Placebo (n=51)	Probiotic (n=53)	OR (95% CI)	p
Achieving MCID n (%)	14 (26)	26 (51)	2.81 (1.15,6.88)	0.023	20 (39)	20 (38)	1.26 (0.51,3.12)	0.623

OR, odds ratio; CI, confidence intervals

4.6 Results: Acceptability

Acceptability outcomes for the diet interventions are presented in **Table 4.11**. A greater proportion of patients reported negative ('no') rather than positive ('yes') responses to diet acceptability, and therefore acceptability questions are presented in the negative context. For most questions, more patients receiving low FODMAP advice found the diet less acceptable than patients receiving sham dietary advice. Regarding the acceptability of being involved in the research overall, over half of the patients reported the benefits of involvement outweighed the burden of following the diet (60% sham diet vs 73% low FODMAP diet, p=0.217).

Table 4.11 Proportion of patients with IBS reporting negative responses to diet acceptability questions (compared with usual diet) who completed a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=95)

Question	Sham diet n=48	Low FODMAP diet n=47	p[#]
Meal preparation was difficult	28 (58)	43 (91)	**<0.001**
Time spent shopping for food was longer	22 (46)	40 (85)	**<0.001**
Time spent preparing and cooking meals and snacks was longer	11 (23)	34 (72)	**<0.001**
Finding suitable food choices when eating out was difficult	38 (79)	45 (96)	**0.027**
The flavour of the meals and snacks was unappealing	8 (17)	37 (78)	**<0.001**
Grocery shopping and eating was more expensive	4 (8)	40 (85)	**<0.001**
Diet was inconvenient	34 (71)	42 (89)	**0.039**
Diet was troublesome/difficult	34 (71)	43 (91)	**0.017**
Made many changes to diet	28 (58)	44 (94)	**<0.001**

Values are n (%) reporting yes/neutral responses; [#]Chi-squared test

A summary of results relating to acceptability of the probiotic are presented in **Table 4.12**. Most patients reported willingness to trial a probiotic in the future (84%). Regarding questions that assessed patient understanding of probiotics, most patients reported they believed they understood the definition of a probiotic prior to the RCT (65%) and most classified a probiotic as a complementary/alternative medicine (53%) rather than standard treatment (21%) or neither complementary medicine or standard treatment (26%). The full acceptability results are presented in Appendix 9.20.

Table 4.12 Proportion of patients reporting responses to product acceptability questions who completed a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=95)

Question	Placebo n=45	Probiotic n=50	p[#]
Taking the sachets daily for 4 weeks was easy	40 (89)	39 (77)	0.169
I experienced a side effect from probiotic/placebo sachet	8 (17)	9 (19)	1.000
I would take a probiotic in the future	41 (92)	38 (76)	0.076

Values are n (%). [#]Chi-squared test

4.7 Discussion and conclusion

4.7.1 GI symptoms and stool output

The aim of this chapter was report the effect of low FODMAP dietary advice and probiotic on clinical outcomes in IBS. The hypothesis that there is a difference between the proportion of patients reporting adequate relief of symptoms between following low FODMAP dietary advice and patients following sham dietary advice can only be tentatively accepted, as there was only a trend towards statistical significance (p=0.057).

The lack of statistically significant difference between dietary intervention groups for the adequate relief endpoint and the disparity with previous data could be explained in a number of ways. Firstly, the proportion of patients achieving adequate relief (57%) was lower than that reported in the only other low FODMAP dietary advice RCT that reported a dichotomous symptom endpoint (68%) (Staudacher et al., 2012). The previous study was essentially unblinded which may have artificially enhanced the response rate in those receiving low FODMAP dietary advice. Secondly, in the current RCT, low FODMAP dietary advice was delivered without explanation of how the diet affects GI physiology, which may be important for its success in enhancing patient 'buy in' to the diet, although this hypothesis requires

formal evaluation. There may have been a greater response rate if this had been included as part of the education process. Finally, although the adequate relief endpoint is currently considered the gold standard for measuring response in IBS trials (Irvine et al., 2006) and has been used as the primary endpoint in series of trials for IBS drugs such as alosetron and tegaserod, its multiple limitations have received significant attention in the literature (described in Section 2.5.1.1).

One recent analysis of pooled linaclotide data in IBS-C showed that a large proportion of non-responders to the dichotomous endpoint in these trials in fact reported some improvement in important clinical symptoms (63% reported improvement abdominal pain and 52% in complete spontaneous bowel movements) (Lacy et al., 2014), which adds to the uncertainty of adequate relief as an appropriate endpoint in IBS. Similar contradictions were found in a recent study of the low FODMAP diet in IBS patients (n=75) where no difference was evident between low FODMAP diet and controls using a dichotomous global symptom endpoint whereas global symptoms scored using VAS were significantly lower after low FODMAP diet (Piacentino et al., 2015). This was also so in the current study in that low FODMAP dietary advice resulted in significant symptom improvement when measured using both the IBS-SSS and the GSRS that did not translate into greater numbers with adequate relief of symptoms. Although adequate relief was chosen as the primary endpoint for the current RCT its usefulness as a primary endpoint in future research requires clarification (see Chapter 7).

Adequate relief was reported in more patients receiving probiotic than those receiving placebo in this RCT. Although this was clearly a statistically significant finding, this RCT was not powered to detect differences for the probiotic intervention. Furthermore, there was a disparity between the outcome for the ITT population and the PP population, suggesting there was a lack of an effect when only those compliant and completed patients in the RCT were considered. Another reason that one should interpret the effect of probiotic on adequate relief with caution is that previous studies of VSL#3 have reported lower response rates using dichotomous endpoints (33%-46%) (Kim et al., 2003, Kim et al., 2005) than found here. Perhaps more importantly, the outcome for adequate relief in this RCT distinctly deviates from findings of the IBS-SSS and GSRS instruments. There was a lack of effect of probiotic on IBS-SSS scores, incidence of symptoms according to the GSRS, severity of symptoms according to the GSRS (except for flatulence severity), and for any stool outcomes. Therefore, taken together, although there appears to be an effect of probiotic on adequate relief here, the global

symptom response rate appears to be substantially higher than previous studies and is at odds with multiple other symptom findings. Therefore the results require replication in a suitably powered study before a definitive conclusion can be made about the effect of VSL#3 on global or individual IBS symptoms.

In stark contrast to the findings for adequate relief, there were wide-ranging significant effects of low FODMAP dietary advice on individual symptoms according to other validated instruments including the IBS-SSS. Three studies have so far measured the effect of low FODMAP dietary advice using the IBS-SSS, all reporting reduction in score (75-130 points), with a difference from baseline (Bohn et al., 2015) and lower scores compared with controls (Harvie et al., 2013, Pedersen et al., 2014). The change in IBS-SSS score demonstrated by the low FODMAP group in this RCT is consistent with previous data and is almost twice that recently reported for a multistrain probiotic preparation (Sisson et al., 2014).

Although the improvement across almost all IBS-SSS subscores in this RCT has not been evident in all previous studies, it is interesting that two of the three previous studies also fail to demonstrate improvement in pain severity subscore (Bohn et al., 2015, Harvie et al., 2013). The reason for this is unclear, especially considering that in the current RCT pain severity according to the GSRS fell in the low FODMAP diet group. This discrepancy may be due to differences in the range of possible answers between the two instruments i.e. four response options for the GSRS on a Likert scale versus many possible responses for the IBS-SSS on a VAS.

The proportion of patients in the low FODMAP group that met the MCID for the IBS-SSS in this RCT is impressive (73%). Unfortunately, comparison with other studies is limited as many fail to report this endpoint. One probiotic study has recently reported 45% of patients met the MCID (Sisson et al., 2014), but there is no previous data available either for patients receiving low FODMAP dietary advice or VSL#3 supplementation. Nevertheless, it is likely that this outcome is very representative of the proportion of patients responding to low FODMAP dietary advice as it is comparable to reported overall response rate from previous trials (Staudacher et al., 2014).

Patients in the low FODMAP diet group demonstrated improvement in the severity of individual symptoms of bloating, pain and flatulence. The beneficial effect of a low FODMAP diet on these symptoms has been shown in previous RCTs using the GSRS (Staudacher et al.,

2012), VAS (Halmos et al., 2014) and IBS-SSS subscores (Bohn et al., 2015). Indeed, a recent systematic review reported that pain and bloating were the most responsive symptoms to low FODMAP dietary intervention (Marsh et al., 2015). This is unsurprising as FODMAP challenge clearly increases colonic fermentation (Ong et al., 2010, Murray et al., 2014) and colonic volume (Major et al., 2015b), and therefore restriction should reduce luminal distension and sensory afferent input induced by gas, thereby improving this cluster of symptoms.

Other symptoms that are probably induced by luminal distension from increased gas production are belching and borborygmi, which both improved in response to low FODMAP advice. These symptoms are less frequently measured in clinical trials, although borborygmi was measured in one study and was responsive to low FODMAP dietary advice (Staudacher et al., 2012). Both cause significant social distress to some patients and should routinely be measured in IBS trials.

The probiotic intervention had an effect on improving flatulence severity compared with placebo in this RCT, in the absence effect on other GI symptoms. This supports data from a previous study where improved flatulence scores were evident after the same dose of VSL#3 after 4 weeks but no effect on bloating or pain (Kim et al., 2005). One other VSL#3 supplementation study in IBS measured flatulence and found no effect of probiotic compared with placebo (Kim et al., 2003), although half the daily dose (450 billion bacteria) was used compared with the other studies. Overall, it appears likely that VSL#3 has an impact on flatulence in IBS, although these results require replication in an adequately powered study.

Diet or product interventions had no impact on symptoms of nausea, heartburn or acid reflux in this RCT. Gastroesophageal reflux disease commonly co-exists with IBS (Whitehead et al., 2007), although the reported incidence and severity of these symptoms at baseline in this RCT was relatively low overall. Tiredness was also unchanged in response to low FODMAP dietary advice. The cause of tiredness is clearly multifactorial and probably varies from one patient to the next. Taking into account that the underlying pathophysiology of upper GI and systemic symptoms is distinct from lower GI symptoms in IBS, it is not unexpected that low FODMAP dietary advice did not improve this cluster of symptoms.

Various symptoms relating to stool evacuation and stool output were measured in this study, including urgency and diarrhoea. There was a beneficial effect of low FODMAP dietary advice

on incidence and severity of urgency in this RCT, which supports results from one previous study that measured this symptom (Staudacher et al., 2012). Urgency is a pervasive problem and can have a major impact on the ability of patients to undertake day-to-day activities, but is unfortunately an infrequently evaluated outcome. The improvement in urgency can be explained by the well-established effect of FODMAPs on increasing ileal water (Barrett et al., 2010, Marciani et al., 2010, Murray et al., 2014, Major et al., 2015b). To this effect, it is surprising that there was a lack of improvement in diarrhoea and/or loose stool symptoms after low FODMAP dietary advice according to the GSRS. Although reduced ileal water is associated with improved perception of stool consistency, at least in ileostomy patients (Barrett et al., 2010), no RCTs until now have in fact demonstrated a positive impact of a low FODMAP diet on reported diarrhoea in IBS patients.

The reduction in incidence and severity of the sensation of incomplete evacuation in patients after low FODMAP dietary advice is somewhat surprising and has not been shown previously. This may be related to evacuation of a firmer stool, leading to a more defined start and end of each stool, or even to reduction in visceral hypersensitivity, which was not measured here. Further work is required to characterise the burden of this symptom in non-constipated patients with IBS, and to measure treatment response. In contrast, there was no effect of low FODMAP dietary advice on improving constipation or hard stools in this RCT. This can be explained by the abovementioned effect of FODMAPs on increasing ileal water, and therefore FODMAP restriction is unlikely to soften hard stool, and may in fact lead to the reverse. However, constipation scores here and in one other RCT that measured it (Staudacher et al., 2012) did not worsen. Furthermore, stool water does not significantly change in any IBS subtype in response to low FODMAP feeding, although this has only been shown in a small subgroup analysis (Halmos et al., 2014). The effect of a low FODMAP diet on stool water, constipation, and indeed for overall symptoms in patients with IBS-C requires further investigation.

Despite no clear effect of low FODMAP dietary advice on diarrhoea or loose stool (measured using the GSRS), a reduction in stool consistency according to the BSFS was apparent, although frequency and proportion of normal stools did not change. Other studies have reported that the low FODMAP diet had an effect on increasing the proportion of normal stools (Staudacher et al., 2012), reducing stool frequency compared with baseline (Bohn et al., 2015) and reducing stool frequency in IBS-D compared with a control diet (Halmos et al., 2014). The reason for the

lack of effect of low FODMAP dietary advice on stool frequency and proportion of normal stools in this RCT is unclear. Different outcomes between studies may be due to a number of reasons including variation in trial methodologies, nuances in the low FODMAP dietary advice provided, differences in IBS subtypes included and/or varying IBS severity inclusion criteria cutoffs.

The effect of the interventions on stool output was also measured in this RCT by the 'satisfaction with bowel habit' IBS-SSS subscore. This question could theoretically encompass any combination of factors related to bowel habit, including stool consistency, frequency, urgency and completeness of evacuation. Patients in the low FODMAP diet group demonstrated an improved subscore compared with those in the sham diet group, in agreement with previous studies (Pedersen et al., 2014, Halmos et al., 2014). This IBS-SSS subscore outcome contrasts with the lack of effect on GSRS scores for diarrhoea and loose stool in this RCT. This might be due to variable attitudes and definitions for the terminology of some of these symptoms, which has been shown to be the case for diarrhoea in non IBS patients (Majid et al., 2012). Overall, it can be concluded that the low FODMAP diet improves overall satisfaction with bowel habits in addition to specific symptoms of urgency, and sensation of incomplete evacuation.

The absence of an interaction between the diet and product interventions for each clinical outcome in this factorial design study infers that the individual effect of each intervention is statistically additive. However, significant effects were not evident between groups when intervention combinations were compared for adequate relief and stool output. Examination of change in IBS-SSS score amongst intervention combinations, in fact, confirmed that low FODMAP dietary advice appeared to be driving most of the differences between intervention combination groups. A parallel trial design planned *a priori* to investigate the clinical effects of a combination low FODMAP diet-probiotic intervention is required to confirm whether the two can indeed be considered additive for clinical outcomes in IBS.

4.7.2 HRQOL

HRQOL determines health seeking behaviour in patients with IBS (Williams et al., 2006), which emphasises the importance of its measurement and identification of treatments that improve HRQOL in this patient group. It is known patients with IBS report poor HRQOL, and even score lower on some HRQOL measures than patients with other chronic disease e.g. end stage renal

disease (Gralnek et al., 2000). Furthermore, measures of HRQOL, rather than GI symptoms alone, may better describe the overall impact of treatment and the shift in illness experience in patients with IBS. This is because HRQOL in IBS is primarily predicted by extraintestinal symptoms such as fatigue and feelings of nervousness, tension and hopelessness rather than physical symptoms (Spiegel et al., 2004). Also, theoretically, the impact of a treatment could be considered a net result of its effect on the condition in question and any disadvantages associated with treatment (e.g. cost, side effects). Accordingly, although low FODMAP dietary advice improved IBS symptoms, the burdens associated with following the diet (as demonstrated by the acceptability data), could influence its ability to positively influence HRQOL.

According to the SF-36 outcomes in this RCT, low FODMAP advice led to significant improvement in some components of generic HRQOL. Specifically, there was an improvement in 'role limitations due to physical health' and 'energy/fatigue'. These outcomes suggest low FODMAP dietary advice led to improvement in the ability to complete work and daily activities in the context of physical health and reduced feelings of fatigue, tiredness and lack of energy. The general health scale score was higher for the low FODMAP group compared with sham but this difference did not reach statistical significance, although it was significant on analysis of the PP population.

Disease-specific measures of quality of life are more responsive to treatment effects than generic measures (Guyatt et al., 1993) and are recommended for evaluating changes in HRQOL in IBS (Bijkerk et al., 2003). This RCT demonstrates that the low FODMAP diet induces significant measurable improvements in IBS-specific HRQOL. These results are in agreement with the improved IBS-SSS subscore for patients in the low FODMAP group relating to quality of life. Indeed, there was a 2.81 higher odds of achieving the MCID for the IBS-QOL in the low FODMAP diet group compared with the sham diet group, and there were significantly higher scores for three specific subscales. Two of these subscales were associated with impact of IBS on relationships with other individuals ('social reaction', 'relationships'). The third subscale, 'body image', included questions relating to 'feeling fat' and being limited with clothing choice. Increased score for this subscale is likely due to parallel improvements in physical GI symptoms e.g. bloating. Food avoidance was the subscale that was scored the lowest across all groups at baseline, suggesting that patients associate dietary factors with their condition. There was no

effect of dietary intervention on the food avoidance subscale, which is unsurprising as participation in the RCT required restriction of the types of foods to be consumed.

The findings for IBS-specific HRQOL in this study replicate data from other low FODMAP studies. For example, a previous unblinded RCT has shown a similar absolute improvement in mean total IBS-QOL score from baseline (approximately 15 points) in patients after 3 months (Harvie et al., 2013), and improvements in IBS-QOL scores are evident in patients in other less robustly designed studies (Mazzawi et al., 2013, Ostgaard et al., 2012, Pedersen et al., 2014). Although many large intervention trials in IBS fail to measure HRQOL which prevents comprehensive comparisons of the effect of low FODMAP dietary advice with other approaches, similar absolute improvement in IBS-QOL score have been shown for antispasmodic medications (Clave et al., 2011, Hou et al., 2014) and cognitive behavioural therapy (Labus et al., 2013). The HRQOL results from this RCT suggest low FODMAP dietary advice can be considered a therapy that, although complex and requiring considerable effort on behalf of the patient, improves HRQOL to at least an equivalent degree to other effective therapeutic approaches.

This is the first study to assess the effect of VSL#3 supplementation on HRQOL using robust validated HRQOL instruments. There was no change in any components of either the generic or IBS-specific HRQOL instruments in patients receiving probiotic compared with those receiving placebo. One study has reported improvement in some aspects of HRQOL in response to VSL#3 supplementation but this was using an unvalidated questionnaire (Michail and Kenche, 2011). The lack of an effect of VSL#3 on HRQOL is unsurprising considering there was no impact on IBS-SSS scores, most GSRS scores and stool outcomes. A trial adequately powered for symptom response is required to confirm whether there is an effect of VSL#3 supplementation on HRQOL in IBS patients.

4.7.3 Acceptability

The acceptability data in this study are useful for two reasons. Firstly, they present information about the overall benefit of the interventions from a patient perspective. This type of data is infrequently measured in nutritional intervention studies (Jackson et al., 2005) but is imperative for planning and development of clinical services. Furthermore, acceptability influences the likelihood of adherence (Berkow et al., 2010), and therefore is an important endpoint for any trials measuring the effect of dietary intervention on disease outcomes.

Overall, a large proportion of patients in the low FODMAP diet group reported the diet was difficult to follow and that many changes were required in order to implement the advice provided. Many patients reported difficulties with practical aspects of the diet, such as time spent preparing meals, eating out, and the cost associated with alternative products. These issues are commonly identified issues in clinical practice, and some have been reported previously in IBD patients following low FODMAP dietary advice (Gearry et al., 2009). With regard to the probiotic intervention, acceptability responses were generally positive, with most patients reporting that the probiotic was easy to take and that they were willing to take a probiotic in the future.

In the unblinded clinical situation difficulties with practical dietary issues (e.g. cooking while on the low FODMAP diet, perceived probiotic side effects) are more easily addressed as time can be spent providing practical solutions for difficulties or barriers specific to each patient. Qualitative studies are required to delve into these issues further in order to improve our understanding of the burdens associated with dietary change, particularly in relation to the low FODMAP diet, and to determine whether strategies currently aimed to help minimise patient burden actually do so.

The second benefit of acceptability data in this RCT is that it helps establish the suitability of the placebo interventions. For example, it is reassuring that responses were equivalent between groups for most product acceptability questions, implying that those that did not receive active intervention had a comparable overall experience to those that received the active product. With regard to the diet acceptability questions, there is a suggestion that aspects of the sham diet could be fine-tuned. There is inherent difficulty in developing a dietary intervention that imposes a significant practical burden on patients whilst causing minimal change in nutrient intake, however these results highlight potential areas of the sham diet that could be addressed in the future, for example, restriction of additional foods that would impact on palatability and time spent on food preparation.

4.7.4 Strengths and limitations

This is the first placebo-controlled RCT investigating the impact of low FODMAP dietary advice on a wide range of IBS symptoms and stool output using a battery of validated symptom measurement instruments. It is likely that application of multiple assessment instruments

better captures the change in symptom experience of patients and also counters potential differences in patient definitions of key symptoms (e.g. diarrhoea). It is also the largest ever RCT investigating the clinical impact of the low FODMAP diet, and the largest to investigate the effect of low FODMAP dietary advice and VSL#3 on generic and disease-specific HRQOL and patient acceptability outcomes. The demographic characteristics of patients recruited to this RCT were representative of the wider population, being predominantly female, mostly IBS-D and of relatively young age (Lovell and Ford, 2012) and therefore the outcomes presented here are considered generalisable to the wider IBS population.

Until now, the only other placebo-controlled blinded RCT investigating the effect of the low FODMAP diet on clinical outcomes provided all food and fluid to patients (Halmos et al., 2015) which arguably hold less external validity than the current RCT. Furthermore, RCTs that have provided patients with low FODMAP dietary advice have either been unblinded (Staudacher et al., 2012, Harvie et al., 2013, Pedersen et al., 2014) or have not been placebo-controlled (Bohn et al., 2015). Therefore, the outcomes presented from this blinded placebo-controlled RCT could be considered among the most robust to date.

Compliance to the interventions in this RCT was very good according to subjective and objective measures of compliance. This not only increases confidence that outcomes of the RCT are due to the interventions rather than external factors, but also confirms that the interventions can be undertaken by patients. Furthermore, outcomes for the ITT and PP populations were generally concordant, which further strengthens confidence in the results (Thabane et al., 2013), and suggests the methodology broadly reproduced aspects of clinical practice. Of the randomised patients, withdrawals totaled 9% of the total recruited sample, which is under the recommended proposed cut off of 20% (Irvine et al., 2006).

This is the first RCT to measure the success of blinding patients to a low FODMAP dietary advice intervention. Although 70% of patients correctly guessed their allocation, this was still fewer than that reported previously in a crossover low FODMAP feeding study (83%) (Halmos et al., 2014). It is acknowledged that successful blinding is rarely achieved in dietary intervention studies, and therefore whether a better outcome than this is possible is uncertain. The product was allocated in a double blind fashion in this RCT, and most patients were unable to guess their allocation (72%), which is better than that reported in another

probiotic RCT (Whorwell et al., 2006), and also confirms the effectiveness of the placebo as a placebo product.

There are also strengths of this RCT that relate to HRQOL and acceptability. This was the first blinded RCT investigating HRQOL in response to low FODMAP dietary advice through the application of commonly used validated HRQOL instruments. This allowed for comparison with previous low FODMAP studies and other non-dietary IBS interventions. Baseline SF-36 and IBS-QOL scores in this RCT were similar to that previously reported in large studies of primary and secondary care patients with IBS (Gralnek et al., 2000, Sisson et al., 2014, Clave et al., 2011) suggesting the impact of low FODMAP dietary advice on HRQOL are generalisable to the wider population with IBS.

There were a number of limitations of the RCT specific to the clinical outcomes presented in this chapter. Firstly, relating to the blinding of patients, the probiotic intervention was allocated in a double-blind fashion however it was impossible to double blind the dietary advice. Therefore, dietary advice was provided in a single blind fashion, with the same conviction and level of detail provided to each group. However, it cannot be guaranteed that there were subtle unconscious differences in the way the advice was presented, which may have led to experimenter bias and contributed to patients becoming suspicious of their allocation. Furthermore, patients, where required, were asked to avoid online searches for dietary treatments for their symptoms to promote continued naivety to the low FODMAP diet. Whether patients complied with this advice is unknown.

Blinding is essentially an attempt to spread expectancy evenly across groups (Colagiuri, 2010). Any unblinding in this RCT may have also occurred in patients who received active treatment and experienced symptom improvement. This is acknowledged as a problem inherent in any trial with physical symptom endpoints and is difficult to address. The application of two blinded interventions in this RCT may have mitigated expectation bias (as if one intervention becomes unblinded the patient is still blinded to the other), however this is impossible to verify. Overall, it is accepted that methodological issues such as blinding and placebo are a particular challenge in dietary intervention studies and may never be fully remedied.

Four other limitations relate to methodological aspects of the RCT. Firstly, there are two issues related to recruitment. Due to time constraints sufficient patients (n=1572) could not be

recruited to power the RCT for detecting differences for adequate relief between probiotic and placebo groups. Furthermore, recruitment across two sites could be considered a disadvantage in this RCT. This relates to the provision of the dietary advice itself, which ideally should be delivered to all recruited patients by the same dietitian. There may have been some variability in researcher bias in this RCT due to two dietitians conducting the study visits and providing the dietary advice. However this was likely to be minimal as only eight patients were recruited at the second site, and the second dietitian was trained and observed to optimise consistency in the way trial visits were conducted to help limit this bias as much as possible.

Secondly, patients recruited for this RCT were a homogenous group of secondary care patients and within a specified age range. Patients with IBS-C and with major comorbidity were excluded. Symptoms at baseline were of moderate severity according to the IBS-SSS, of low to moderate severity according to the GSRS, and HRQOL scores were comparable to IBS patients from primary and secondary care. Therefore, although these symptom, HRQOL and acceptability outcomes are likely applicable to most IBS patients, whether they are generalisable to patients in primary care, IBS-C patients, those with significant comorbidity, or those experiencing symptoms at the mild or severe extremes of symptom severity cannot be confirmed.

Thirdly, the dietary advice provided in this RCT was not completely representative of the style of delivery in the clinical situation. Specifically, in order to prevent unblinding, advice to the low FODMAP diet group did not include an explanation of the physiological basis of the low FODMAP diet. This may have underestimated the true effect of the diet that might be possible in real life practice. Alternatively, in clinical practice patients do not usually undertake as intensive evaluation, are not telephoned weekly and do not provide stool samples and therefore the effect of merely participating in a study may have overestimated the true effect of the diet compared with real life practice.

Finally, there were limitations related to the measurement of the clinical endpoints. There is an inherent difficulty in measuring GI symptoms in IBS due to the lack of available biomarkers for symptom severity. Limitations of these instruments have been discussed in detail elsewhere (Section 2.5 and Section 4.7.1). Until more objective measures of IBS symptoms are accepted, large blinded RCTs that use robust validated methods of measuring patient reported outcomes are essential. The problem of reactivity, where participation in research leads to

behaviour change (i.e. the Hawthorne effect), is also relevant when considering the measurement of clinical endpoints. For example, involvement in the trial could lead to increased attention to other lifestyle factors which might impact on experience or perception of IBS symptoms and clinical outcomes (Kramer and Shapiro, 1984). However, this effect should be even across groups in a large enough trial, and therefore should not have influenced outcomes of this RCT.

4.7.5 Significance of results

Clinical benefit of low FODMAP dietary advice on IBS symptoms over and above placebo has never been investigated. This placebo-controlled RCT provides evidence that low FODMAP dietary advice leads to improvement in overall and specific GI symptoms in patients with non-constipation predominant IBS. Specifically, 73% of patients report a global clinical response based on the IBS-SSS, and 57% report response based on the adequate relief question, although the potential limitations of the latter endpoint are acknowledged. At a conservative estimate, this corresponds to a 2-3 greater odds of symptomatic response to low FODMAP dietary advice compared with placebo, which is at least equivalent to some pharmaceutical treatments (e.g. some antispasmodics, antidepressants) (Enck et al., 2010). With regards to specific symptoms, it is likely that patients with bloating, borborygmi, flatulence and dissatisfaction with bowel habit (specifically urgency and sensation of incomplete evacuation) will benefit from this intervention. Although the diet may be onerous with regards to cost, palatability and the extra time required, clinical response is paralleled with improvements in a number of HRQOL outcomes. This confirms that reductions in physical symptoms as a result of the low FODMAP diet translate into improvement at a broader biopsychosocial level. In contrast, the effect of VSL#3 on symptoms and HRQOL in IBS is equivocal. There was clear disagreement between global measures of response (adequate relief vs IBS-SSS) for patients receiving probiotic in this RCT and a wide-ranging lack of response for other clinical variables measured. Although any potential benefit of this probiotic can be considered additive when used as a parallel treatment with low FODMAP dietary advice, its benefit as a sole therapy is unconvincing based on these results, and a suitably powered study is required to confirm this finding.

4.7.6 Conclusion

This RCT has demonstrated that dietitian-led low FODMAP dietary advice provides a therapeutic effect for a wide range of IBS symptoms in a majority of patients compared with placebo, which is known to be a powerful phenomenon in IBS. Furthermore, symptom improvement is associated with increased HRQOL scores, confirming there is a beneficial impact on patient-perceived health despite the burdensome nature of the diet. This is a key finding considering that treatment of IBS is often suboptimal and less than 40% of patients in report satisfaction with treatment (Drossman et al., 2009). Whether the improved HRQOL in response to low FODMAP dietary advice translates into reduced healthcare utilisation or wider economic implications (e.g. reduced absenteeism, reduced requirement for medication) is unknown and requires evaluation.

Reduced ileal water and colonic fermentation are the likely mechanisms by which the low FODMAP diet improves GI symptoms. Further work is required to broaden our understanding of the effects of the diet on stool consistency and other aspects of stool output, and indeed its overall effectiveness in IBS-C. In addition to the effects on clinical parameters, the low FODMAP diet may lead to consequences such as altered nutrient intake, changes in the GI microbiota and other aspects of the colonic environment. These will be addressed in Chapters 5 and 6.

5 The effect of low FODMAP dietary advice on nutrient and FODMAP intake in irritable bowel syndrome

5.1 Introduction

Dietary management of IBS with the low FODMAP diet may be effective in a large proportion of patients with IBS. In Chapter 4 it was demonstrated that 57% of patients reported adequate relief of GI symptoms and 73% of patients achieved the MCID for IBS-SSS, however one potential shortcoming of many exclusion diets is the risk of nutritional inadequacy, and this is especially so if the excluded foods are nutrient-rich. A gluten free diet, for example, leads to a reduction in carbohydrate, fibre and a number of B vitamins in females, and a reduction in iron intake in males and females compared with the pre-diagnosis diet (Shepherd and Gibson, 2013). Likewise, lower micronutrient intake (calcium, iron, folate) has been reported in those with atopic dermatitis following an exclusion diet compared with controls (Kim et al., 2013), and patients with multiple food allergies have a lower intake of protein and a number of micronutrients (including B vitamins) when compared with patients requiring only single food exclusion (Kim et al., 2013). Although research in other disease groups is helpful in estimating the potential risk associated with an exclusion diet in IBS, nutrient intake data may be confounded by the effect of the disorder itself, and of course the risk is specific to the features of the diet in question.

The effect of short term exclusion of high FODMAP foods on nutrient intake has been investigated in two dietary advice RCTs (Bohn et al., 2015, Staudacher et al., 2012). Only one of these included an evaluation of micronutrient intake (iron, calcium). In a small sample of patients with IBS, there was a lower calcium intake in those following low FODMAP advice compared with controls consuming their habitual diet (603 mg vs 730 mg, p=0.016) (Staudacher et al., 2012). This is a preliminary finding and more extensive evaluation is required in a larger cohort. In addition, this finding was a comparison between the low FODMAP diet and usual diet and therefore it is unclear whether this was the result of any dietary exclusion or specific to the low FODMAP diet. Furthermore, whether micronutrient intake of patients with IBS on a low FODMAP diet are comparable with the general UK population and meet UK dietary reference values (DRVs) has not been reported.

5.1.1 Aim of this chapter

The aim of this chapter is to report the results of a RCT to investigate the effect of low FODMAP dietary advice on nutrient and FODMAP intake in patients with IBS. The chapter will be divided into three parts:

1. Inter-rater agreement between diet record coders (Section 5.2.1)
2. Habitual nutrient intake of patients with IBS (Section 5.2.2)
3. Compliance with the dietary interventions (Section 5.2.3)
4. Nutrient intake results for the low FODMAP group and sham diet group from the RCT of low FODMAP dietary advice (Section 5.2.4)
5. Bodyweight (Section 5.2.5)

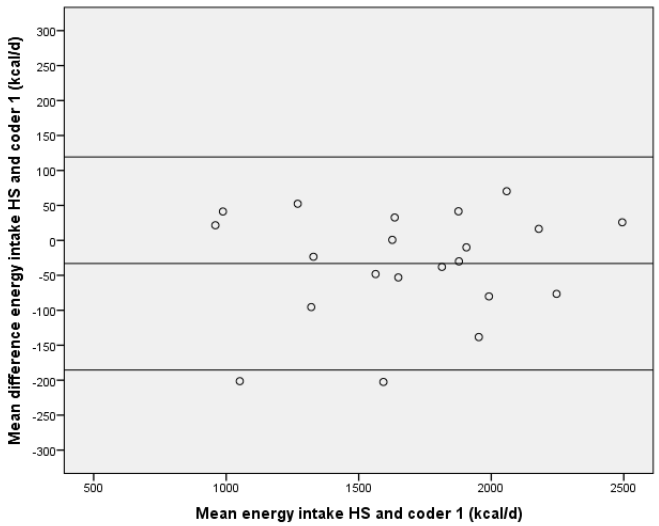
5.2 Results

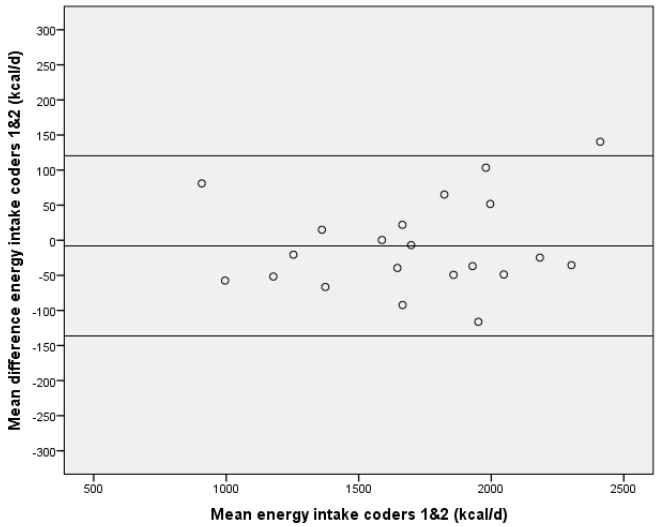
Diet records were coded and entered into dietary composition software by HS and two additional dietitian coders. A total of 199 7-day diet records were entered by three coders. HS entered 20 diet records and the remainder were coded by the two other coders. Inter-rater agreement was evaluated as described in Section 2.7.5.

5.2.1 Inter-rater agreement

Inter-rater agreement was evaluated for energy and NSP for the first 21 diet records available (7 baseline, 7 sham diet, 7 low FODMAP diet), and therefore these were coded in triplicate. Bland-Altman plots were examined (see **Figure 5.1** for energy and Appendix 9.21 for NSP).

Most differences between coders were within the limits of agreement of mean difference ± 1.96 SD. The mean differences for daily energy and NSP intake are presented in **Table 5.1** and were <50 kcal/d for energy and <1 g/d for NSP. Intraclass correlation coefficients were greater than 0.9 indicating excellent reliability between coders. A total of nine outliers (where mean difference > 1.96 SD) were identified and it was noted that these outliers were due to various discrepancies which were categorised. These were attributed to: patient error (unclear portion size or food description), coder error (portion size error, omission, food code error), database error. The database error was due to duplication of an existing database food with a newly created identical food that was different in NSP content. These errors were corrected prior to final nutrient intake analysis presented in the following sections.





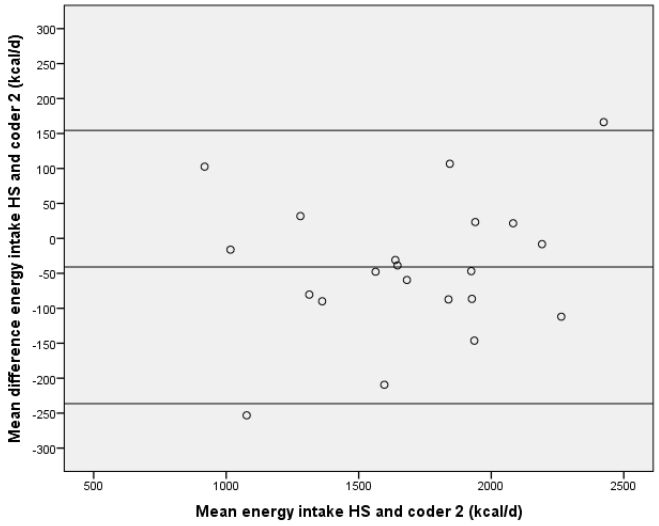


Figure 5.1 Bland Altman plots for energy intake from 21 diet records (7 baseline, 7 sham diet, 7 low FODMAP diet) to assess inter-agreement between three diet record coders. Records were from patients with IBS participating in a 2x2 factorial design RCT of the low FODMAP dietary advice and probiotic supplementation

Table 5.1 Mean difference and intraclass correlation coefficients between three coders for energy and NSP for 21 diet records (7 baseline, 7 sham diet, 7 low FODMAP diet) to assess inter-agreement. Diet records were from patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

Coders	Energy (kcal/d)		NSP (g/d)	
	Mean difference (95% CI)	Intraclass correlation coefficient	Mean difference (95% CI)	Intraclass correlation coefficient
HS and Coder 1	-33 (-68, 2)	0.990	-0.2 (-0.9, 0.4)	0.982
Coder 1 & Coder 2	-8 (-38,22)	0.994	-0.3 (-1.1, 0.4)	0.980
HS & Coder 2	-41 (-86, 4)	0.984	-0.1 (-0.8, 0.6)	0.978

Values are mean difference and 95% confidence intervals. NSP, non starch polysaccharides

5.2.2 Habitual nutrient intake of patients with IBS

Habitual diet of all patients in the RCT was assessed by comparing nutrient intake at baseline with gender-specific nutrient DRVs (Department of Health, 2004) and national population intakes in age and gender-matched individuals in the UK (Public Health England and Food Standards Agency, 2014) (Appendix 9.22). Nutrient intake at baseline on average met requirements for most nutrients, and was similar to that of national population intakes. Mean daily intake of calcium for males (909 mg/d) and females (790 mg/d) was higher than the reference nutrient intake (RNI, 700 mg/d). In males (n=34), intake of iron (12.4 mg/d) was higher than the RNI (8.7 mg/d), whereas in 19-49 year old females (n=70), iron intake (11.0 mg/d) was lower than the RNI (14.8 mg/d) and in females intake of potassium (2759 mg/d) was lower than the RNI (3500 mg/d).

5.2.3 Compliance with the dietary interventions

Self-reported compliance to the dietary interventions measured at weekly telephone visits was very good. All patients were classified as compliant to the dietary advice, where noncompliance was defined as following the diet <50% of the time on at least 2 of the 4 assessments. Most patients reported following the diet at the highest rating of compliance ('followed the diet >75% of the time') at each week. There was no difference in compliance between groups (**Table 5.2**).

Table 5.2 Proportion of patients with IBS who completed the 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation and reported the highest rating of compliance ('I followed the diet more than 75% of the time') (n=95)

	Sham diet n=48	Low FODMAP diet n=47	p[#]
Week 1 n (%)	46 (96)	44 (94)	0.884
Week 2 n (%)	45 (93)	43 (92)	0.784
Week 3 n (%)	43 (90)	43 (92)	0.668
Week 4 n (%)	44 (92)	41 (88)	0.548

[#]Chi-squared test

Total and individual FODMAP intake from diet records was evaluated (**Table 5.3**). In the low FODMAP diet group, there was a lower intake of total and individual FODMAPs at follow up compared with baseline ($p<0.05$), except for total and excess fructose ($p>0.05$). FODMAP intake was compared between diet groups at follow up after adjusting for baseline differences. There was a significantly lower intake of total FODMAPs in the low FODMAP diet group compared with the sham diet group (9.9 g/d vs 17.4 g/d, $p<0.001$) and lower intake of fructans, sorbitol, lactose (all $p<0.001$) and mannitol ($p=0.041$), but no difference between groups for GOS (0.8 g/d vs 0.9 g/d, $p=0.080$) or excess fructose (1.9 g/d vs 1.4 g/d, $p=0.821$).

5.2.4 Nutrient intake results from the RCT of low FODMAP dietary advice

Energy, macronutrient, NSP and micronutrient intake from food and fluid were evaluated at baseline and follow up in the RCT. Fifteen micronutrients considered potentially at risk on restriction of FODMAPs were included in the analysis (potassium, calcium, magnesium, phosphorous, iron, zinc, beta-carotene, B vitamins and vitamin C).

A total of 27 (26%) patients reported intake of a vitamin or mineral supplement (31% sham diet, 21% low FODMAP diet) at baseline. Multivitamin and mineral supplements were the most commonly reported (13% of all patients), followed by vitamin C (7%), iron (6%) and calcium (3%). Two patients were strict lacto-ovo vegetarians (1 sham, 1 low FODMAP). Results are presented for the ITT population for nutrient contribution from food and fluid only, the most conservative estimate of dietary intake of the cohort.

Table 5.3 Total and individual FODMAP intake for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet n=53			Low FODMAP diet n=51			$p^{##}$	Change between baseline and follow up		$p^{###}$
	Baseline	Follow up	$p^{\#}$	Baseline	Follow up	$p^{\#}$		Sham diet	low FODMAP diet	
Total FODMAPs (g/d)*	17.6 (8.8)	17.4 (10.5)	0.527	18.3 (8.7)	9.9 (6.4)	**<0.001**	**<0.001**	-0.2 (7.8)	-8.4 (9.1)	**<0.001**
Fructans (g/d)	5.0 (2.6)	5.0 (2.9)	0.403	5.0 (2.0)	2.5 (1.9)	**<0.001**	**<0.001**	-0.1 (2.9)	-2.5 (2.8)	**<0.001**
GOS (g/d)	0.9 (0.5)	0.9 (0.5)	0.730	0.8 (0.5)	0.8 (0.6)	**0.018**	0.080	-0.01 (0.6)	-0.1 (0.6)	0.490
Lactose (g/d)	9.0 (7.8)	8.9 (9.1)	0.843	9.4 (7.7)	4.3 (4.3)	**<0.001**	**<0.001**	-0.1 (6.2)	-5.2 (7.7)	**<0.001**
Total fructose (g/d)	14.3 (7.7)	15.5 (8.7)	0.314	13.4 (6.3)	12.7 (5.9)	0.347	0.112	1.3 (6.2)	-0.8 (7.0)	0.121
Excess fructose (g/d)	1.4 (1.2)	1.4 (1.4)	0.286	2.0 (2.0)	1.9 (2.8)	0.134	0.821	-0.1 (0.4)	-0.2 (7.8)	0.614
Sorbitol (g/d)	1.0 (1.0)	1.0 (1.0)	0.688	0.7 (0.8)	0.3 (0.5)	**<0.001**	**<0.001**	0.04 (1.1)	-0.4 (0.8)	**0.031**
Mannitol (g/d)	0.4 (0.3)	0.3 (0.3)	0.051	0.4 (0.4)	0.1 (0.2)	**<0.001**	**0.041**	-0.06 (0.4)	-0.2 (0.4)	**0.025**

Values are mean (SD). Data were log transformed

*Total FODMAPs are calculated as the sum of individual carbohydrates including excess fructose (not total fructose)

$^{\#}$ Dependent samples t-test baseline vs follow up within groups $^{##}$ANCOVA follow between groups adjusted for baseline $^{###}$Independent samples t-test

5.2.4.1 Nutrient intake in the low FODMAP group

The impact of low FODMAP dietary advice on nutrient intake was assessed by comparing nutrient intake at follow up in the low FODMAP diet group with baseline (habitual diet) (**Table 5.4**). There were no differences in energy, protein, fat or NSP intake between baseline and follow up in the low FODMAP group. Daily NSP intake for patients in the low FODMAP diet group at baseline and follow up is presented in **Figure 5.2**.

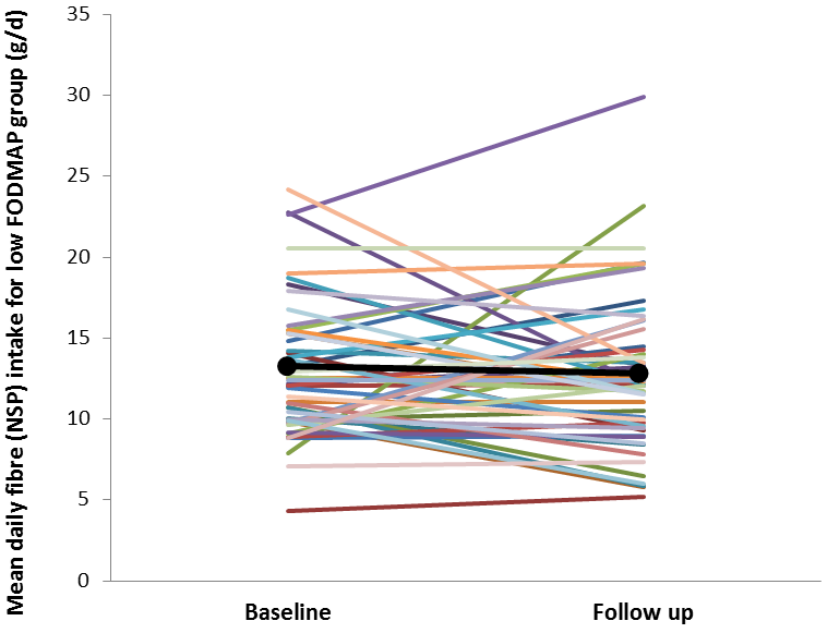


Figure 5.2 Mean daily NSP intake for individual patients at baseline and follow up in the low FODMAP group (n=51) from the 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation No significant differences in NSP intakes were detected (13.0 g/d vs 12.8 g/d; p=0.578)

In the low FODMAP diet group, there was a lower intake of total carbohydrate at follow up compared with baseline (198 g/d vs 213 g/d, p=0.020) and for starch (110 g/d vs 122 g/d, p=0.004). There was no difference in intake of micronutrients at follow up except for iron which was lower compared with baseline (10.3 mg vs 11.3 mg, p=0.009), and for beta-carotene and vitamin B6 which were higher compared with baseline.

Table 5.4 Total energy and nutrient intake from food and fluid for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet n=53			Low FODMAP diet n=51			P[##]	Change between baseline and follow up		p[###]
	Baseline	Follow up	p[#]	Baseline	Follow up	p[#]		Sham diet	low FODMAP diet	
Energy, macronutrients, NSP										
Energy (kcal/d)	2042 (504)	1891 (599)	**0.013**	1957 (525)	1861 (465)	0.079	0.517	-139 (395)	-78 (312)	0.386
Protein (g/d)	80 (20)	75 (21)	**0.041**	77 (25)	78 (22)	0.772	0.100	-5 (16)	1 (14)	0.306
Fat (g/d)	85 (24)	80 (28)	0.114	81 (24)	78 (25)	0.298	0.787	-5 (21)	-3 (19)	0.652
Carbohydrate (g/d)	229 (62)	206 (62)	**<0.001**	213 (58)	198 (58)	**0.020**	0.678	-23 (45)	-15 (45)	0.380
Starch (g/d)	128 (40)	115 (39)	**0.001**	122 (35)	110 (39)	**0.004**	0.916	-13 (28)	-12 (30)	0.850
Sugars (g/d)	90 (33)	84 (33)	0.134	82 (29)	75 (30)	0.058	0.457	-6 (27)	-6 (23)	0.893
NSP (g/d)	14.5 (5.9)	13.3 (5.1)	**0.038**	13.0 (4.2)	12.8 (4.8)	0.771	0.578	-1.2 (4.1)	-0.2 (4.6)	0.223
Micronutrients										
Potassium (mg/d)	3026 (945)	2851 (873)	0.059	2915 (871)	3006 (745)	0.231	**0.029**	-175 (661)	92 (539)	**0.026**
Calcium (mg/d)	860 (347)	803 (292)	0.159	797 (275)	773 (316)	0.346	0.769	-57 (288)	-23 (176)	0.480
Magnesium (mg/d)	290 (110)	267 (89)	**0.002**	275 (91)	272 (78)	0.707	**0.045**	-32 (71)	-3 (53)	**0.019**
Phosphorous (mg/d)	1311 (368)	1206 (342)	**0.005**	1240 (385)	1227 (343)	0.707	0.112	-106 (262)	-13 (241)	0.063
Iron (mg/d)	11.9 (3.7)	11.4 (4.0)	0.208	11.3 (3.1)	10.3 (2.5)	**0.009**	0.157	-0.5 (2.8)	-1.0 (2.6)	0.338
Zinc (mg/d)	9.0 (2.8)	8.1 (2.6)	**0.004**	8.5 (2.7)	8.6 (2.7)	0.779	0.072	-0.9 (2.1)	0.1 (2.9)	**0.044**
Beta-carotene (µg/d)	3800 (3081)	3878 (2655)	0.834	3777 (2795)	5027 (3632)	**0.025**	**0.044**	78 (2701)	1250 (3848)	0.074

Values are mean (SD). [#] Dependent samples t-test baseline vs follow up within groups [##]ANCOVA follow between groups adjusted for baseline [###]Independent samples t-test change between groups

Table 5.4 (cont) Total energy and nutrient intake from food and fluid for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet n=53			Low FODMAP diet n=51			$P^{##}$	Change between baseline and follow up		$P^{###}$
	Baseline	Follow up	$P^{\#}$	Baseline	Follow up	$P^{\#}$		Sham diet	low FODMAP diet	
Thiamin (mg/d)	1.5 (0.5)	1.4 (0.4)	**0.027**	1.4 (0.4)	1.3 (0.4)	0.460	0.604	-0.1 (0.3)	0 (0.4)	0.351
Riboflavin (mg/d)	1.6 (0.7)	1.5 (0.6)	0.100	1.5 (0.6)	1.5 (0.5)	0.194	0.879	-0.1 (0.4)	-0.1 (0.5)	0.906
Niacin (mg/d)	20.6 (7.6)	19.7 (7.5)	0.283	21.0 (8.5)	21.7 (8.7)	0.383	0.120	-0.9 (5.9)	0.6 (4.8)	0.167
Vitamin B6 (mg/d)	2.0 (0.7)	1.9 (0.6)	0.272	1.9 (0.7)	2.1 (0.6)	**0.036**	**0.016**	-0.1 (0.5)	0.1 (0.5)	**0.023**
Vitamin B12 (µg/d)	5.2 (2.5)	4.7 (2.3)	0.115	5.3 (3.8)	6.1 (2.6)	0.064	**0.001**	-0.5 (2.4)	0.9 (3.2)	**0.018**
Folate (µg/d)	226 (90)	230 (92)	0.640	234 (93)	235 (83)	0.920	0.980	3 (56)	1 (69)	0.840
Pantothenic acid (mg/d)	4.9 (1.8)	4.5 (1.5)	**0.043**	4.7 (1.7)	4.8 (1.9)	0.718	0.091	-0.4 (1.5)	0.1 (1.3)	0.073
Biotin (µg/d)	35 (17)	31 (18)	**0.014**	35 (18)	35 (17)	0.820	0.079	-3.6 (10.4)	0.5 (15)	0.112
Vitamin C (mg/d)	93 (51)	91 (48)	0.758	95 (55)	109 (58)	0.053	**0.039**	-2 (37)	14 (52)	0.073

Values are mean (SD). $^{\#}$ Dependent samples t-test baseline vs follow up within groups, $^{\#\#}$ANCOVA follow between groups adjusted for baseline $^{\#\#\#}$Independent samples t-test change between groups

In order to evaluate the nutritional adequacy of the low FODMAP diet, intake of macronutrients and micronutrients in the low FODMAP group at follow up were compared with gender-specific DRVs and intake in age and gender-matched individuals in the UK (Appendix 9.22). Broadly, mean nutrient intakes met or exceeded the DRVs for most nutrients and intakes were broadly similar to that of UK individuals. For females (n=35), mean daily intake of potassium (2876 mg/d) was lower than the RNI (3500 mg/d) and in 19-49 year olds iron intake (10.7 mg/d) was lower than the RNI (14.8 mg/d).

Nutrient intake of patients in the low FODMAP diet group was also examined by comparing the proportion of patients meeting the NSP individual minimum intake (the NSP equivalent for RNI, which will now be referred to as RNI) and RNI for micronutrients (for which an RNI is available) at follow up compared with baseline (**Figure 5.3**). The proportion of patients meeting the RNI was significantly lower at follow up compared with baseline for calcium (49% vs 65%, p=0.039) and for iron, although the latter did not reach statistical significance (43% vs 49%, p=0.375). There were no significant differences in gender-specific comparisons with the DRVs (Appendix 9.23).

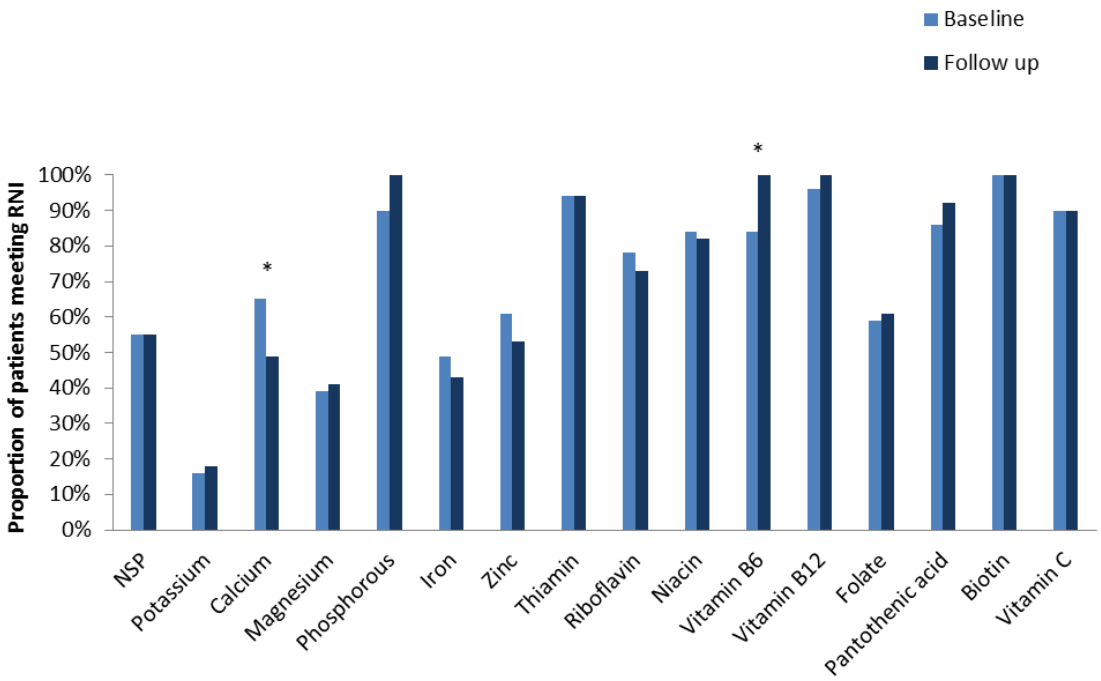


Figure 5.3 Proportion of patients meeting RNI at baseline and at follow up in the low FODMAP diet group (n=51) in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation * p<0.05 McNemar's test

5.2.4.2 Nutrient intake in the low FODMAP diet group compared with the sham diet group

Nutrient intake was compared between the low FODMAP group and the sham diet group at follow up (**Table 5.4**). There were no significant differences between the two diet groups for energy or macronutrient intake after adjusting for baseline differences. Neither were there any differences in micronutrient intake between the two diet groups at follow up except for a higher intake of potassium, magnesium, beta-carotene, some B vitamins and vitamin C in the low FODMAP diet group.

5.2.4.3 Nutrient and FODMAP intake in the sham diet group

The impact of sham dietary advice on nutrient intake was assessed by comparing intake at follow up with baseline (habitual diet) in the sham diet group (**Table 5.4**). There was a lower intake of energy at follow up compared with baseline (1891 kcal/d vs 2042 kcal/d, p=0.013), as well as for carbohydrate (206 g/d vs 229 g/d, p<0.001), starch (115 g/d vs 128 g/d, p=0.001), protein (75 g/d vs 80 g/d, p=0.041) and NSP (13.3 g vs 14.5 g/d p=0.038). There were also lower intakes of magnesium, phosphorous, zinc, pantothenic acid and biotin. Proportions of patients meeting the RNI for NSP was not different at follow up compared with baseline (66% vs 60%, p=0.549). Total and individual FODMAP intake did not change (**Table 5.3**).

5.2.5 Bodyweight

In the sham diet group, bodyweight at follow up was not significantly different to baseline (72.5 kg vs 72.7 kg, p=0.259). In the low FODMAP diet group, bodyweight was lower at follow up compared with baseline (68.7 kg vs 69.1 kg, p=0.026), however there was no difference in bodyweight change between the sham diet and low FODMAP diet groups (-0.2 kg vs -0.4 kg, p =0.480).

5.3 Discussion and Conclusion

The aim of this chapter was to investigate the effect of low FODMAP dietary advice on nutrient intake in patients with IBS. A 7-day unweighed diet record was used to measure dietary intake in this RCT, and is the most accurate method of evaluating dietary intake other than the gold standard weighed diet record (Bingham et al., 1995). The diet records in this RCT also included portion size photographs to assist patients with portion size estimation (Bingham et al., 1994). Three coders entered the diet record data for analysis and inter-rater agreement analysis suggested excellent agreement between coders. Hence, these results are a robust and

accurate representation of the diet and nutrient intake of a large cohort of patients with IBS recruited from secondary care.

5.3.1 Habitual nutrient intake of patients with IBS

This study demonstrates that nutrient intake of patients with IBS consuming their habitual diet generally meets national recommendations. Iron intake in females of 19-49 years of age was lower than the RNI, however, this is also evident in the wider UK female population (Public Health England and Food Standards Agency, 2014). Potassium intake in females was also lower than the RNI. This might be attributable to a lower overall intake of fruit and vegetables (which are good sources of potassium) among individuals with IBS, however the intake of potassium reported here was higher than the UK general population. Therefore these data suggest there is no evidence of IBS-specific nutrient inadequacy when patients follow their habitual diet. The current data supports previous work in UK community patients with IBS (n=104) which showed that habitual intake met the RNIs for macronutrients and a small number of evaluated micronutrients (Williams et al., 2011). The same has been reported for secondary care patients with IBS in Sweden (n=187) in which intake was evaluated using 4-day diet records and all 14 micronutrients analysed met the Nordic dietary intake recommendations (Bohn et al., 2013).

Habitual nutrient intake of patients in this study was broadly similar to gender-matched individuals from the UK population (Public Health England and Food Standards Agency, 2014). This supports previous data from our group that has demonstrated nutrient intake of UK secondary care patients with IBS was similar to healthy controls (McCoubrey et al., 2008). Intake of calcium and iron in that study was lower in patients compared with controls, however, which might be related to differences in dietary assessment methodology. Indeed, FFQs, have been shown to underestimate calcium intake, and were used to evaluate intake in that study (Bingham et al., 1994).

This is the largest UK study to evaluate habitual dietary intake in IBS using diet records, including assessment of the intake of a large range of micronutrients. Mean nutrient intakes generally met RNIs and reflected nutrient intake of the general UK population. This is a valuable contribution to the existing literature as previous studies have been limited by their use of FFQ (McCoubrey et al., 2008, Williams et al., 2011), which can over or under-estimate intake of some nutrients (Bingham et al., 1994), or use unvalidated questionnaires to assess dietary intake (Hayes et al., 2014). Furthermore, many studies do not provide sufficient detail

on the specific dietary data collection method, food composition databases used or dietary coding protocols.

It is acknowledged that external validity of this study may be limited somewhat due to the likelihood of some responder bias (i.e. those that were recruited to the RCT may be more attentive to their diet). Furthermore, this group of patients had been referred to secondary care and therefore their dietary intake may not be representative of community patients with IBS, although it could be hypothesised that secondary care patients are likely to have more restricted diets. Overall, these results are reassuring considering up to 92% of secondary care patients with IBS change their dietary intake in an attempt to alleviate their symptoms and less than 15% do so with formal dietetic advice (Hayes et al., 2014).

5.3.2 The effect of low FODMAP dietary advice on nutrient intake

According to subjective and objective compliance measures, patients in the low FODMAP group adhered well to the dietary advice in this RCT. Subjective compliance was better than that reported previously for patients following the low FODMAP diet using a similar 4-point subjective scoring system (Shepherd and Gibson, 2006), although this is expected as the current study was of much shorter duration (4 weeks vs 14 months) and it is recognised that self-reported compliance reduces over time spent following a diet (Berkow et al., 2010).

Total FODMAP intake reduced by approximately half in the low FODMAP group in this RCT, and absolute intake at follow up was approximately half of the total FODMAP intake reported by patients in the sham diet group. This magnitude of difference is similar to that in patients following low FODMAP dietary advice compared with a control group consuming habitual diet in a previous RCT (Staudacher et al., 2012). However, the reduction in FODMAP intake identified here is smaller compared with a recent Swedish dietary advice study where 4-day d records reported a quartering of FODMAP intake compared with baseline (4 g/d vs 17 g/d) (Bohn et al., 2015). This disparity in FODMAP intake is somewhat expected due to differences in habitual FODMAP intake secondary to cultural differences, likely variability in the dietary advice provided, and differences in food composition tables used to analyse dietary intake.

There was a significant difference between low FODMAP diet and sham diet groups in the intake of total and most individual FODMAPs at follow up after adjustment for baseline differences. This confirms the effectiveness of low FODMAP advice in reducing FODMAP intake

in this RCT. There was no difference between low FODMAP diet and sham diet groups for GOS intake at follow up, although the low FODMAP diet group did demonstrate a reduction from baseline whilst intake in the sham group remained stable. Both groups had relatively low intake of GOS at baseline when compared with previous data (Staudacher et al., 2012), which may have contributed to the lack of difference at follow up. There was a lack of difference between groups at follow up for intake of excess fructose, and this has been evident in other work (Bohn et al., 2015). Fructose in excess of glucose at a sitting, rather than the total excess fructose over a day, is more important for determining whether fructose is incompletely absorbed. Therefore the results for excess fructose here are not of concern.

Adherence to the low FODMAP diet requires a reduction in the intake of energy dense foods (e.g. milk, cereals, pulses, nuts). This RCT provides evidence that intake of protein and fat in patients following low FODMAP dietary advice is not different to habitual dietary intake. One potential risk of a low FODMAP diet is a reduction in energy intake due to inadequate substitution of high FODMAP foods, which was evident in a recent Swedish study, where daily energy intake reduced by more than 400 kcal in patients following low FODMAP dietary advice compared with baseline (Bohn et al., 2015). It was not reported whether this resulted in any change in bodyweight. In the current RCT, there was a small reduction in energy intake (approximately 100 kcal/d) in the low FODMAP diet group which was accompanied by a reduction in mean bodyweight of 0.4 kg over the duration of the 4-week intervention. Total energy intake was not statistically different to baseline, similar to a previous UK dietary advice RCT (Staudacher et al., 2012), and suggests that patients were generally able to substitute sufficient alternative foods into the diet, under the instruction of a skilled dietitian, to maintain energy intake. Whether bodyweight is maintained after four weeks, during the reintroduction of FODMAPs and in the longer term requires investigation.

Intake of carbohydrate and starch reduced in the low FODMAP diet group compared with baseline, in line with previous research (Staudacher et al., 2012). A gluten free diet leads to a reduced intake of polysaccharide carbohydrates (De Palma et al., 2009) and the low FODMAP diet similarly involves a restriction of wheat and gluten-containing products. Some patients may not choose to include wheat free alternative breads and cereals due to poor palatability and/or higher cost, which might have contributed to these findings. Nevertheless, intake of carbohydrates at follow up in the low FODMAP group in this study (198 g) still represented a much higher intake than that used in a low carbohydrate intervention in IBS (20 g) (Austin et

al., 2009) and the difference compared with baseline could be considered nutritionally insignificant, and unlikely to impact on microbiota outcomes.

Fibre can improve or worsen IBS symptoms (Eswaran et al., 2013) and also has effects on the microbiota and markers of fermentation (Whelan et al., 2005). Therefore, in order to investigate the independent effect of restricting fermentable carbohydrates in IBS, it was essential that overall fibre intake was maintained in the low FODMAP diet group. Despite alterations in the intake of total FODMAPs, total carbohydrate and starch, NSP intake was maintained in the low FODMAP diet group. This was evident both when evaluating mean daily intake as well as the proportion of patients meeting the RNI. Previous low FODMAP dietary advice studies have either shown maintenance of fibre intake (Staudacher et al., 2012) or a reduction (Bohn et al., 2015), whilst feeding studies attempt to maintain fibre intake by addition of fibre supplements to low FODMAP meals (Barrett et al., 2010, Ong et al., 2010). Whether changes occurred to the intake of soluble or insoluble fibre fractions cannot be ruled out, as the current dietary analysis software did not allow for their evaluation, however it is unlikely that there were dramatic differences in the context of an overall preservation of total NSP intake.

This is the most comprehensive evaluation of micronutrient intake in patients following low FODMAP dietary advice. Other low FODMAP dietary advice studies have either only evaluated macronutrient intake (Bohn et al., 2015) or measured intake of a limited number of micronutrients (e.g. iron, calcium) (Staudacher et al., 2012). Intake of all micronutrients except for iron was maintained from baseline in the low FODMAP diet group. Although the difference at follow up was not different to the sham group after adjusting for baseline, this may still represent a nutritional concern, and a trend for a reduced iron intake in patients following the low FODMAP diet has been demonstrated previously (Staudacher et al., 2012).

A reduction in iron intake could plausibly occur due to substitution of iron-fortified wheat breakfast cereals (iron content 7-13 mg/100g) with low iron non-wheat products such as oats (0.5 mg/100g), or a reduction in the intake of pulses (2-3 mg/100g). However, examination of the proportion of patients achieving the RNI for iron may be more indicative of meaningful changes in dietary iron intake as mean daily intakes can be skewed by a small number of individuals with extreme values. The proportion of patients in the low FODMAP diet group meeting the RNI for iron was not different at follow up compared with baseline, and this was

also the case when gender-specific groups were examined. Finally, although there was an overall reduction in iron intake amongst patients in the low FODMAP diet group, a small reduction was also evident in the sham diet group. Therefore, it could be the change in iron intake in both groups was due to transient dietary change in response to being observed, and may not represent an effect specifically related to following a low FODMAP diet.

Calcium is one micronutrient that has been considered at potential risk of deficiency in patients following a low FODMAP diet, and has previously been shown to be reduced compared with controls following their habitual diet (Staudacher et al., 2012). Intake of calcium for the low FODMAP diet group (773 mg/d) was not significantly lower than habitual diet (797 mg/d) in this RCT, and was higher than previous data (603 mg/d) (Staudacher et al., 2012) and on average exceeded the RNI (700 mg/d). However on examination, calcium was the only micronutrient for which there was a significantly lower proportion of patients meeting the RNI at follow up compared with baseline, which is equivalent to 8/51 patients failing to meet the RNI after low FODMAP advice although they met the RNI while following their habitual diet.

The likeliest explanation for the effect of low FODMAP dietary advice on calcium intake is an inadequate substitution of high lactose calcium-rich foods with low lactose calcium-rich alternatives (e.g. lactose free milk products or calcium-fortified plant milks). It could also be due to natural variation in dietary intake (Bingham, 1987), however the likelihood of this being the case is small, particularly given the large sample size. Another potential important contributor to this finding is measurement error resulting from the lack of nutrient composition data for novel lactose free products. Where possible, such novel foods were registered as new foods on the analysis database and their calcium content added, where available. Calcium is essential for a number of physiological functions including nerve conduction and hormone secretion, and inadequate intake may be related to osteoporosis, cardiovascular disease and other chronic conditions (Peterlik and Cross, 2009). If there is a true reduction in calcium intake when patients follow the low FODMAP diet, this may have long term ramifications, especially if the diet is followed strictly for extended periods of time.

This RCT demonstrates that patients following a low FODMAP diet broadly maintain nutrient intake despite being advised to restrict intake of many nutrient-rich food items across a number of food groups. Furthermore, where RNIs were not met, they also failed to be met

whilst patients were consuming their habitual diet. This suggests that advice from a specialist dietitian regarding a low FODMAP diet does not broadly compromise nutrient intake and that the low FODMAP diet could potentially be used without nutritional concern. However, these results confirm that there is risk of patients failing to meet the RNI for calcium and this requires specific attention in the clinical consultation.

5.3.3 The suitability of the sham diet

An important outcome of this study was the nutrient intake of patients following sham dietary advice. Compliance data for the sham diet indicates patients followed the diet as well as those patients in the low FODMAP diet group. Objective measurement of compliance was difficult, as there was no specific measurable food component that could be used to evaluate this. Arguably, in this situation, the fact that patients perceived they were following a 'special' diet was more important than compliance to the diet itself.

The aim of the sham diet was to modify dietary carbohydrate sources and restrict an equivalent number of foods compared with the low FODMAP diet but to have no impact on nutrient or FODMAP intake. A reduction in energy, carbohydrate, protein and NSP was evident in the sham diet group compared with baseline. However, when comparing intake at follow up with the low FODMAP diet group, and when comparing the change in nutrient intake between groups, there were no differences. Importantly, the proportion of patients achieving the RNI for NSP at baseline compared with follow up did not change. Fibre can worsen or improve symptoms, and therefore it was vital that fibre intake was preserved during the sham diet for it to be considered a 'placebo diet'. There were some differences in micronutrient intake in the sham diet group, however this is unlikely to have any impact on nutritional status in the short term or on clinical or microbiological outcomes in the RCT.

Another key aim of the sham diet was that it did not alter FODMAP intake. Total and individual FODMAP intake clearly was maintained in the sham diet group, and was comparable to previous data from patients with IBS following habitual intake (Staudacher et al., 2012, Bohn et al., 2015). There was a trend for a reduced intake of mannitol in the sham group, which may have been due to a reduced intake of high mannitol vegetables, however this is unlikely to have had any effect on clinical outcomes.

Overall, the results of the dietary intake analysis of patients in the sham group have demonstrated that the sham diet developed for this RCT was entirely suitable as a placebo comparator for low FODMAP dietary advice in terms of its nutritional composition. Alteration in intake of energy, some macronutrients and micronutrients was evident, however the change in intake of these nutrients between groups was not significantly different for most nutrients, and would not have had any bearing on clinical or microbiological outcomes.

5.3.4 Strengths and limitations

This is the largest study that has prospectively assessed habitual dietary intake of UK patients with IBS and the most comprehensive dietary evaluation of patients following low FODMAP dietary advice. It is also the first to assess the effect of low FODMAP dietary advice on dietary intake in the context of a placebo-controlled study and the first to evaluate the effect of a novel sham diet on dietary intake in IBS.

When individuals are asked to record food and fluid intake there is risk of them altering their intake in order to simplify the process or to impress the researcher (Bingham, 1987). Comparison of low FODMAP dietary advice with a placebo dietary advice intervention somewhat accounts for this response bias and might allow for a more precise estimate of important alterations in nutrient intake that might occur external to the research setting. It is acknowledged that assessment of dietary intake is subject to error, however it was endeavoured to minimise this where possible. The assessment methodology (7-day diet record) chosen is a robust method for measuring dietary intake whilst minimising patient burden. Significant effort was made to ensure all diet records were checked and clarified with each patient, and verification of inter-rater agreement between coders maximised the accuracy of coding.

The study has some limitations that need to be acknowledged. This study included patients between ages 18-65 years, without significant co-morbidity or significant existing dietary restrictions, and only non IBS-C subtypes were included. Therefore whether these outcomes can be extrapolated to other patient groups is unclear. There was also likely to be a degree of response bias leading to inclusion of patients with a keen interest in dietary approaches to managing their symptoms, which may have underestimated the true effect of the low FODMAP diet on nutrient intake.

Furthermore, this study examined dietary intake in the fourth week following low FODMAP dietary advice. Nutrient intake in patients following low FODMAP advice for extended periods has not been prospectively investigated. Hypothetically, calcium intake could improve over time as patients adjust and adapt to their new dietary regime or are able to reintroduce some lactose into the diet. The converse could be also be true, that calcium intake might reduce as patients become less enthusiastic about incorporating variety into the diet. Finally, it must be acknowledged that there is limited availability of alternative food composition data. This may have led to inaccuracy and/or underestimation of nutrient intake, which adds to the inherent difficulties of measuring dietary intake using food tables.

5.3.5 Significance of the results

This study has confirmed that low FODMAP dietary advice leads to a clear reduction in FODMAP intake but does not significantly alter energy or nutrient intake in patients with IBS-D, IBS-M and IBS-U, other than for calcium in some patients. This is important for clinical practice as many patients will follow the low FODMAP diet for at least four weeks. The low FODMAP diet led to a minor reduction in bodyweight over the 4-week period, although the change in bodyweight was not different to that which occurred in the sham diet group, and therefore this is unlikely a clinical concern for most patients, but emphasises the importance of monitoring anthropometric outcomes in routine clinical care. NSP intake in the low FODMAP diet group was maintained and therefore any clinical or luminal alterations that occur in patients in this RCT in response to a low FODMAP diet can confidently be attributed to the restriction of FODMAPs rather than changes to NSP intake.

The sham diet designed for use as a placebo comparator intervention in this study proved to be successful in altering food intake without altering macronutrient or total FODMAP intake compared with the low FODMAP diet group. Some differences were evident for micronutrient intake, which is unsurprising given the difficulty in designing such a diet, and this is unlikely to have any impact on other outcomes of this RCT. Furthermore, this is a valuable resource that can be utilised in future studies that examine clinical response to dietary intervention in IBS.

Finally, this study has confirmed that habitual dietary intake of patients with IBS is generally nutritionally replete. Mean dietary intake of the nutrients that failed to meet gender-specific RNIs (e.g. iron) were also evident in the general population. Whether the same is the case for

primary care patients, patients with IBS-C or those with multiple comorbidities is not known and requires further investigation.

5.3.6 Conclusion

This RCT has confirmed that comprehensive low FODMAP dietary counselling from an experienced dietitian leads to a reduction in FODMAP intake but does not lead to nutritional implications in secondary care patients with IBS after four weeks, other than for calcium. These results are reassuring considering this dietary intervention requires avoidance of multiple foods across a number of food groups and exclusion diets have been shown to lead to nutritional inadequacy (Kim et al., 2013). It is recommended dietitians place strong emphasis on the inclusion of high calcium foods when advising patients to follow a low FODMAP diet. Whether the results from this study are valid for less compliant patients, patients that are self-taught, or patients advised by non-dietetic healthcare professionals requires investigation.

6 The effect of low FODMAP dietary advice and probiotic supplementation on the gastrointestinal microbiota and markers of fermentation in irritable bowel syndrome

6.1 Introduction

The importance of the GI microbiota in health and disease has become a growing focus of scientific research. Dysbiosis is proposed as one likely contributor to the pathophysiology of IBS. Commonly reported alterations include reduced stool Bifidobacteria and Bacteroidetes concentration and lower diversity compared with healthy individuals (Section 1.1.7.4).

Independent of disease-associated dysbiosis, other external factors have important impact on modulating the abundance and taxonomic composition of the microbiota. For example, habitual dietary intake modulates GI microbiota composition, and acute dietary change can directly influence the microbiota within a short period of time (1-3 days) (Wu et al., 2011, Walker et al., 2011, David et al., 2014). Much of the research that has assessed the effect of carbohydrate-modified interventions on the composition of the microbiota has been conducted in the overweight or obese consuming very low levels of carbohydrate intake (<25 g/d) (Duncan et al., 2007, Russell et al., 2011b, Brinkworth et al., 2009). The effect of carbohydrate-modified dietary intervention on the GI microbiota in IBS has not been extensively studied.

A low FODMAP diet reduces the abundance of GI Bifidobacteria in adults with IBS (Halmos et al., 2015, Staudacher et al., 2012). It may also have effects on other bacterial groups such as *F. prausnitzii*, overall microbiota abundance and luminal fermentation byproducts (Halmos et al., 2015) although this has not been consistently demonstrated (Staudacher et al., 2012). Having lower microbial richness (Tap et al., 2015) or GI symptoms (Manichanh et al., 2014) is associated with increased susceptibility to diet-induced alteration in the microbiota. Therefore, the impact of dietary manipulation on the microbiota in patients with IBS may be more pronounced than in healthy individuals, which is of further concern as this patient group already exhibit dysbiosis (Section 1.1.7.4). The nature and extent of microbiota modulation and its byproducts in response to a low FODMAP diet, and potential approaches to preventing it, requires further evaluation.

The Bifidobacteria-lowering effect of the low FODMAP diet is a concern. Firstly, this genus has a number of beneficial impacts on the host including pathogen exclusion and immunomodulatory effects (Lee and O'Sullivan, 2010). Secondly, there is growing evidence for the inverse relationship of lower luminal Bifidobacteria with clinical symptoms in IBS (Jalanka-

Tuovinen et al., 2011, Rajilic-Stojanovic et al., 2011). Therefore, maintenance of Bifidobacteria may be important for long term GI health and for optimising symptoms in IBS.

Probiotic supplementation can modify the GI microbiota and some products have demonstrated beneficial effect for IBS (Ford et al., 2014b), although which individual species and strains are most effective is unclear. Until now, there has been no study of the use of a low FODMAP diet and probiotic co-administration. A large parallel design study is required to confirm the extent and nature of the effect of the low FODMAP diet on the GI microbiota and SCFA, and to evaluate whether these acute alterations can be prevented by probiotic supplementation.

6.1.1 Aim of this chapter

The aim of this chapter is to report the results for the GI microbiota, SCFA and pH from the 2x2 factorial design RCT investigating the effect of low FODMAP dietary advice and probiotic supplementation in patients with IBS.

The GI microbiota was measured using qPCR (Section 2.6.2), SCFA were measured using GLC (Section 2.6.3), and stool pH was measured using a pH electrode (Section 2.6.4).

The hypothesis was that there is a difference in stool Bifidobacteria concentration between patients following low FODMAP dietary advice and taking a probiotic supplement compared with patients following low FODMAP dietary advice alone (low FODMAP diet + placebo) (Section 2.2).

The chapter is divided into the following two parts:

1. GI microbiota (Section 6.2.1)
2. Stool SCFA and pH (Section 6.2.2)

6.2 Results

For all outcomes in this chapter, there was no interaction between the diet and product interventions and therefore the main effects for these are presented individually, and are adjusted for baseline values. The primary analysis based on the ITT dataset is presented. The analysis for absolute and relative abundance of the microbiota based on the PP population is presented in Appendix 9.24.

6.2.1 GI microbiota

Absolute abundance of total bacteria (universal) and 11 bacterial groups are presented in **Table 6.1**. Linear regression established that diet could predict absolute abundance of four bacterial groups at follow up. Absolute Bifidobacteria abundance could be significantly predicted $F(3,96)=30.709$ ($p<0.001$) by both diet and by product, and they accounted for 47% of the explained variability. Lower absolute abundance was evident in the low FODMAP diet group compared with sham diet ($p=0.028$) and there was higher abundance for probiotic compared with placebo ($p=0.021$).

Diet could predict concentrations of Bacteroides spp. $F(3,98)=39.017$ ($p<0.001$), accounting for 53% of the explained variability, with higher abundance in the low FODMAP diet group compared with the sham diet group ($p=0.040$). Roseburia spp. & *Eubacterium rectale* was also predicted by diet $F(3,89)=40.585$ ($p<0.001$), accounting for 54% of the explained variability, with lower abundance in the low FODMAP diet group compared with sham ($p=0.034$). There were no differences for absolute abundance of any other bacteria measured, including for *F. prausnitzii*.

Results for the relative abundances of the microbiota are presented in **Table 6.2**. Linear regression established that diet could predict relative abundance of Bacteroides spp. $F(3,98)= 38.24$ ($p<0.001$), with a higher proportion in the low FODMAP diet group compared with sham ($p=0.001$). Total Bifidobacteria $F(3,96)=20.11$ ($p<0.001$), *B. longum* $F(3,89)=10.60$ ($p<0.001$) and *B. adolescentis* $F(3,55)=18.012$ ($p<0.001$) were all predicted by diet and were lower in the low FODMAP diet group compared with sham ($p<0.05$). All outcomes remained when the per protocol population were examined except that the absolute abundance of Bacteroides spp. became nonsignificant for diet.

Table 6.1 Absolute abundance of microbiota (log_{10} cells/g faeces) at follow up for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Diet				Product			
	Sham diet (n=53)	**Low FODMAP diet (n=51)**	**Mean difference (95% CI)**	**p**	**Placebo (n=51)**	**Probiotic (n=53)**	**Mean difference (95% CI)**	**p**
Universal	10.9 (0.4)	10.9 (0.3)	-0.04 (-0.17, 0.07)	0.511	10.9 (0.3)	10.9 (0.4)	-0.02 (-0.13, 0.09)	0.741
Bacteroides spp.	10.0 (0.6)	10.1 (0.7)	0.187 (0.02, 0.35)	**0.040**	10.2 (0.5)	10.1 (0.8)	-0.16 (-0.31, 0.01)	0.077
Prevotella spp.	7.8 (1.9)	7.1 (1.7)	-0.23 (-0.56, 0.08)	0.176	7.5 (1.8)	7.4 (1.9)	-0.01 (-0.29, 0.29)	0.953
Bifidobacteria	9.0 (1.1)	8.8 (0.7)	-0.32 (-0.60, -0.03)	**0.028**	8.7 (1.1)	9.1 (0.7)	0.31 (0.03, 0.56)	**0.021**
B. longum	8.5 (0.9)	8.0 (0.8)	-0.60 (-0.82, -0.35)	**0.001**	8.3 (1.0)	8.2 (0.9)	-0.23 (-0.47, -0.01)	0.058
B. adolescentis	8.2 (1.1)	7.6 (1.0)	-0.39 (-0.80, -0.01)	0.066	7.8 (1.1)	8.0 (1.1)	-0.16 (-0.57, 0.24)	0.457
Clostridium Cluster XIVa	10.1 (0.8)	10.2 (0.6)	0.04 (-0.21, 0.27)	0.752	10.1 (0.7)	10.2 (0.7)	0.07 (-0.16, 0.31)	0.597
Roseburia spp. & *E. rectale*	9.8 (0.5)	9.4 (0.8)	-0.19 (-0.38, -0.21)	**0.034**	9.5 (0.8)	9.6 (0.6)	0.01 (-0.18, 0.20)	0.932
F. prausnitzii	9.7 (0.6)	9.5 (0.8)	-0.09 (-0.31, 0.15)	0.469	9.6 (0.8)	9.7 (0.6)	0.09 (-0.19, 0.34)	0.538
R. Bromii	8.7 (0.7)	8.5 (0.9)	-0.05 (0.27, 0.18)	0.672	8.6 (0.8)	8.6 (0.8)	-0.07 (-0.30, 0.17)	0.546
A. muciniphila	7.9 (1.1)	7.9 (1.0)	-0.42 (-0.90, 0.03)	0.074	7.9 (1.0)	7.8 (1.1)	-0.20 (-0.59, 0.19)	0.353
Lactobacilli	7.6 (0.9)	7.6 (0.9)	0.18 (-0.33, 0.68)	0.533	7.5 (1.0)	7.6 (0.9)	0.10 (-0.43, 0.66)	0.680

Values are mean (SD), estimated mean difference and 95% confidence intervals

Table 6.2 Relative abundance of microbiota (% of total) at follow up for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Diet				Product			
	Sham diet (n=53)	Low FODMAP diet (n=51)	Mean difference (95% CI)	p	Placebo (n=51)	Probiotic (n=53)	Mean difference (95% CI)	p
Bacteroides spp.	20 (15)	30 (17)	8.94 (4.67, 13.43)	**0.001**	26 (16)	23 (17)	-3.81 (-8.33, 0.29)	0.088
Prevotella spp.	8 (16)	5 (12)	-2.52 (-8.67, 3.78)	0.433	6 (12)	7 (16)	4.66 (-0.090, 10.31)	0.145
Bifidobacteria	4 (5)	2 (2)	-2.21 (-3.36, -1.19)	**0.003**	3 (5)	3 (3)	0.11 (-1.15, 1.20)	0.867
B. longum	1 (2)	<1 (1)	-0.93 (-1.56, -0,39)	**0.014**	1 (2)	1 (1)	-0.35 (-0.93, 0.18)	0.258
B. adolescentis	1 (1)	<1 (1)	-0.52 (-0.84. -0.22)	**0.018**	<1 (1)	1 (1)	0.03 (-0.25, 0.36)	0.852
Clostridium Cluster XIVa	24 (17)	25(13)	0.92 (-4.35, 6.51)	0.744	22 (16)	26 (15)	4.83 (-0.18, 9.52)	0.062
Roseburia spp. & *E. rectale*	9 (8)	7 (8)	-0.59 (-3.30, 2.16)	0.687	7 (6)	9 (9)	1.70 (-1.21, 4.50)	0.241
F. prausnitzii	9 (9)	9 (10)	0.07 (-3.86, 3.66)	0.974	9 (9)	10 (9)	0.55 (-2.94, 4.38)	0.760
R. Bromii	1 (1)	1 (1)	0.36 (-0.01, 0.72)	0.073	1 (1)	1 (1)	-0.33 (-0.71, 0.06)	0.110
A.muciniphila	<1 (<1)	<1 (1)	0.10 (-0.28, 0.62)	0.722	<1 (1)	<1 (<1)	-0.42 (-0.94, 0.03)	0.254
Lactobacilli	<1 (1)	<1 (<1)	-0.02 (-0.35, -0.18)	0.914	<1 (1)	<1 (<1)	-0.13 (-0.53, 0.12)	0.522

Values are mean (SD), estimated mean difference and 95% confidence intervals

Absolute abundances of microbiota were compared between combination intervention groups (Appendix 9.25). Bifidobacteria abundance was highest in the sham diet + probiotic group and lowest in the low FODMAP diet + placebo group, and this difference was statistically significant on between groups comparisons (9.2 vs 8.6 $\log_{10}$ cells/g; p=0.020). Bifidobacteria concentration was not different between low FODMAP diet + probiotic (8.9 $\log_{10}$ cells/g) and the sham diet + placebo group (8.8 $\log_{10}$ cells/g) but was higher for the low FODMAP diet + probiotic group (8.9 $\log_{10}$ cells/g) compared with the low FODMAP diet + placebo group (8.6 $\log_{10}$ cells/g), although this did not reach statistical significance.

Baseline absolute abundance of Bifidobacteria was compared with change in Bifidobacteria abundance for the per protocol population with detectable Bifidobacteria concentration at both timepoints (**Figure 6.1**). There was a significant negative correlation between baseline Bifidobacteria abundance and change in Bifidobacteria abundance for those receiving low FODMAP dietary advice with placebo or probiotic and for patients receiving sham dietary advice and probiotic (p<0.001) (i.e. those with high baseline Bifidobacteria had a greater reduction in Bifidobacteria), but not for patients receiving sham dietary advice and placebo (p=0.059).

6.2.2 Stool SCFA and pH

Stool SCFA concentrations and pH are presented in **Table 6.3** and the full dataset is presented in Appendix 9.26. Linear regression analysis demonstrated that diet could predict acetate concentration F(3,100)=7.39 (p<.001), accounting for 16% of the explained variability. Acetate was lower in the low FODMAP diet group compared with sham (p=0.029). There was no effect of diet or product on total SCFA, any other SCFA or stool pH, although a trend for lower total SCFA was evident in the low FODMAP group compared with sham diet (p=0.061). The PP analysis revealed no other differences.

When assessing the effect of combination interventions, there was a consistent pattern of a higher concentration of total SCFA, acetate, butyrate and propionate in the low FODMAP + probiotic group compared with the low FODMAP diet + placebo group, but lower concentrations compared with the sham diet + probiotic group, although there were no statistically significant differences (Appendix 9.27).

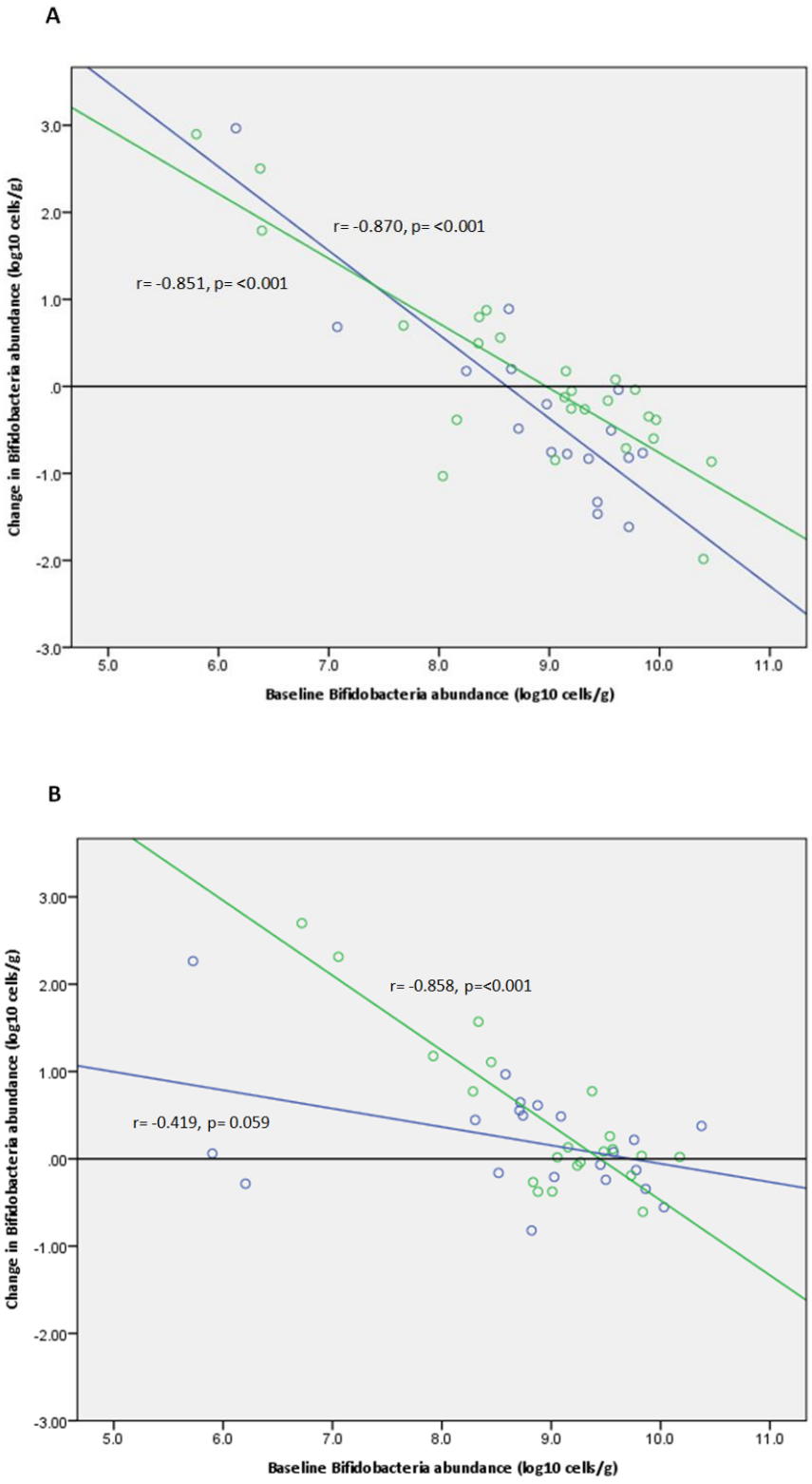


Figure 6.1 Baseline stool Bifidobacteria abundance compared with change in Bifidobacteria abundance for the per protocol population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation A: low FODMAP diet (n=42); B: sham diet (n=42). Values in green represent patients randomised to probiotic and values in blue represent patients randomised to placebo

Table 6.3 Stool SCFA concentration (µmol/g faeces) and stool pH at follow up for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Diet				Product			
	Sham diet n=53	**Low FODMAP diet n=51**	**Mean difference (95% CI)**	**p**	**Placebo n=51**	**Probiotic n=53**	**Mean difference (95% CI)**	**p**
Total SCFA	124.0 (69.4)	98.6 (43.7)	-22.2 (-46.9, -4.0)	0.061	108.6 (42.8)	113.1 (71.9)	10.1 (-9.4, 33.2)	0.374
Acetate	73.1 (37.1)	58.2 (25.9)	-14.5 (-27.3, -3.2)	**0.029**	64.8 (27.4)	66.8 (43.6)	5.3 (-5.0, 17.2)	0.351
Butyrate	21.5 (15.8)	15.7 (9.5)	-3.3 (-7.3, 0.3)	0.120	17.9 (10.1)	19.4 (15.9)	2.7 (-1.4, 7.2)	0.274
Propionate	20.9 (16.2)	18.0 (9.7)	-2.7 (-7.8, 1.5)	0.322	18.9 (8.3)	20.1 (17.0)	2.1 (-2.3, 7.4)	0.439
Valerate	2.4 (1.5)	2.0 (1.3)	-0.4 (-0.8, 0.1)	0.126	2.2 (1.2)	2.2 (1.6)	0.1 (-0.4, 0.5)	0.880
Isobutyrate	2.7 (1.8)	2.6 (1.4)	-0.3 (-0.7, 0.2)	0.302	2.7 (1.7)	2.6 (1.6)	-0.1 (-0.5, 0.4)	0.844
Isovalerate	2.2 (1.4)	2.0 (1.1)	-0.1 (-0.9, 0.3)	0.449	2.2 (1.2)	2.1 (1.4)	-0.1 (-0.8, 0.5)	0.647
pH	6.7 (0.5)	6.8 (0.5)	-0.03 (-0.20, 0.14)	0.181	6.8 (0.5)	6.8 (0.5)	0.7 (0.47, 0.85)	0.737

Values are mean (SD), estimated mean difference and 95% confidence intervals

6.3 Discussion and Conclusion

6.3.1 GI microbiota

This 2x2 factorial design RCT confirmed that low FODMAP dietary advice leads to distinct alterations in the composition of the stool microbiota in patients with IBS. This included a reduction in the absolute and relative abundance of the genus Bifidobacteria and the species *B. longum*, and a reduction in the relative abundance of *B. adolescentis*. At the genus level, the relative abundance of Bifidobacteria across all timepoints (2-4%) was in line with previous data using qPCR on stool samples in IBS (Kerckhoffs et al., 2009), and the alterations in Bifidobacteria induced by low FODMAP dietary advice supports data from previous work using both FISH (Staudacher et al., 2012) and qPCR (Halmos et al., 2015). Moreover, this is the first time that low FODMAP diet-induced species-specific reductions in Bifidobacteria have been reported.

The reduction in Bifidobacteria abundance in the low FODMAP group is most likely explained by the reduced intake of prebiotic fructans and GOS. Compared with patients in the sham diet group, patients in the low FODMAP diet group had lower daily intake of fructans (5 g vs 2.5 g, $p<0.001$) and a trend for lower intake of GOS (0.8 g vs 0.9 g, $p=0.080$), which is in line with previous low FODMAP dietary advice studies (Staudacher et al., 2012, Bohn et al., 2015). These prebiotic carbohydrates are known to enhance abundance of luminal Bifidobacteria when supplemented for four weeks even in small doses (2.5-3.5 g/d) in healthy volunteers (Bouhnik et al., 2007) and in patients with IBS (Silk et al., 2009). This RCT indicates that the reverse is true when a similar absolute quantity is removed from the diet.

The difference in Bifidobacteria abundance in the low FODMAP diet group compared with the sham diet group in this RCT (0.2 $\log_{10}$ cells/g faeces) was less substantial than found previously (0.8 $\log_{10}$ cells/g faeces) (Staudacher et al., 2012). This could be due to methodological differences in microbiota quantification (qPCR vs FISH) but also to differences in the nature of the control groups. Unlike the previous study, the control group in the current study was required to alter their dietary intake (albeit with an aim to maintain nutrient and FODMAP intake) which may have led to a change in Bifidobacteria abundance and therefore reduced the degree of difference between the two groups.

The change in Bifidobacteria concentration was negatively correlated with baseline abundance for most patients in this RCT. Specifically, those that received low FODMAP dietary advice who

harboured higher initial baseline Bifidobacteria (and thus the greatest potential for reduction) demonstrated a greater reduction, and the nature of the association was not different in those who also received probiotic. Likewise, those who received probiotic supplementation alone (probiotic + sham diet) with initial low Bifidobacteria abundance demonstrated the greatest increase in abundance. This inverse association has previously been shown with FODMAP restriction (Staudacher et al., 2012) and with prebiotic supplementation (Whelan et al., 2005) but never in response to probiotic supplementation. Interestingly, there appeared to be a threshold at a baseline Bifidobacteria concentration of 9.0-9.5 $\log_{10}$ cells/g at which probiotic supplementation had no further effect on increasing Bifidobacteria abundance. Whether this is a real phenomenon and whether it occurs with other probiotics in healthy individuals or in IBS requires confirmation.

Although the restriction of the prebiotic fructans and GOS likely had the strongest influence on reducing Bifidobacteria in the low FODMAP diet group, restriction of other dietary constituents may also have contributed to this finding. Wheat (Windey et al., 2014), wholegrain products (Costabile et al., 2008), a range of fruits and nuts and possibly phenolic compounds (Parkar et al., 2013) are bifidogenic and/or impact on other bacterial groups (Graf et al., 2015) and a gluten free diet leads to a reduction in Bifidobacteria and *B. longum* (De Palma et al., 2009). Carbohydrate restriction also leads to reduction in the stool Bifidobacteria (Brinkworth et al., 2009, Duncan et al., 2007) although previous studies severely restrict intake to much lower daily intakes than in this RCT. Nevertheless, although prebiotic restriction likely had a strong influence on the Bifidobacteria abundance for the low FODMAP group here and in previous work, it cannot be ruled out that inevitable alteration in consumption of other dietary constituents also contributed.

It was not unexpected that absolute abundance of luminal Bifidobacteria was higher after four weeks in the probiotic group compared with placebo. VSL#3 is a high dose Bifidobacteria-containing probiotic and its viability in stool has previously been confirmed (Brigidi et al., 2003). There was no effect of probiotic on concentrations of *B. longum* and *B. adolescentis* compared with placebo in this RCT. This is surprising considering that VSL#3 contains both of species. This suggests there were significant shifts in other Bifidobacteria species, such as *B. longum and B. breve*, not measured here due to poor qPCR amplification. The absence of an enhanced Lactobacillus concentration in response to the probiotic is also unexpected considering VSL#3 also contains four Lactobacillus species. On examination of the data,

however, there was an increased concentration of Lactobacillus in the placebo group at follow up which may have contributed to the nonsignificant finding between groups. There was a lower proportion of patients with Lactobacillus below the detection limit in the probiotic group (9%) vs placebo group (53%; $p<0.001$) (Appendix 9.28) indicating that more patients harboured detectable levels of Lactobacillus in the probiotic group compared with placebo.

When combination interventions were compared, low FODMAP dietary advice with probiotic co-administration (low FODMAP diet + probiotic) led to conservation of total Bifidobacteria, evidenced by no difference in abundance compared with the sham diet and placebo group, and the mean absolute abundance was higher compared with the low FODMAP and placebo group, although the latter did not reach statistical significance. Strictly speaking, the hypothesis that there is a difference in the luminal Bifidobacteria concentration between patients following a low FODMAP diet with probiotic compared with patients following a low FODMAP diet alone cannot be accepted. However, these results suggest that the Bifidobacteria-lowering effect of a low FODMAP diet is somewhat ameliorated by VSL#3, and co-administration leads to Bifidobacteria abundance that is at least equivalent with patients who receive neither intervention, which is in agreement with the anticipated outcome presented in Chapter 1 (**Figure 2.2**).

Absolute abundance of Roseburia spp. and *E. rectale*, a subgroup of Clostridium Cluster XIVa, was lower in the low FODMAP diet group compared with the sham diet group in this RCT. The relative abundance of these organisms are similar to that found in other work in obese and healthy individuals (Duncan et al., 2007). These saccharolytic species are able to degrade a wide range of carbohydrates, especially starch (Flint et al., 2012). Total carbohydrate and starch intake were marginally reduced in both low FODMAP diet and sham diet groups, suggesting that the reduction in Roseburia spp. & *E. rectale* abundance in the low FODMAP group may be secondary to reduced colonic availability of short-chain fermentable carbohydrates rather than total carbohydrate or starch. Reduced Roseburia spp. may also at least in part be explained by reduced acetate availability, as this SCFA is known to be a requirement for growth of this group (Duncan et al 2004).

The Roseburia spp. finding here is in line with other work that has shown reduction in abundance of this group on extreme restriction of total carbohydrate (Russell et al., 2011b, Duncan et al., 2007), and in the absence of changes in the larger Clostridium Cluster XIVa to

which it belongs (Duncan et al., 2007). Comparative work also reports a positive association of Roseburia in vegetarians but negative association in omnivores (De Filippis et al., 2015), suggesting that modest differences in carbohydrate intake may be enough to alter abundance of this group. Surprisingly, a low FODMAP feeding study failed to demonstrate differences in Roseburia compared with habitual diet (Halmos et al., 2015). This may be due to the previously mentioned methodological limitations of the study (e.g. pooling of samples), the smaller sample studied, and the addition of psyllium and resistant starch to the low FODMAP diet (that does not occur in routine clinical practice), supplementation of which have been shown to increase the abundance of this group (Walker et al., 2011).

Although the low FODMAP diet group demonstrated a reduction in the abundance of Roseburia spp., a bacterial group that are major butyrate producers, there was no alteration in total stool butyrate concentration compared with sham diet. Carbohydrate restriction has previously shown to lead to a 30-70% reduction in stool Roseburia spp. and *E. rectale* abundance which was positively associated with stool butyrate concentration (Duncan et al., 2007). The absence of a change in stool butyrate is reassuring, and suggests there was sufficient flux through butyrate-producing pathways from other organisms such as *F. prausnitzii*, which has been correlated with relative stool butyrate concentration in healthy individuals consuming a normal diet (Benus et al., 2010). However, the reliance on stool measures of SCFA is limited and will be discussed.

There was a higher abundance of Bacteroides spp. at four weeks in the low FODMAP group compared with sham diet in this RCT. However, on closer inspection, there were within-group reductions in absolute and relative Bacteroides abundance in both diet groups from baseline values (Appendix 9.29). Therefore this finding suggests there was a less marked decline in Bacteroides spp. abundance in the low FODMAP diet group compared with that found for the sham diet group. The decline in Bacteroides in both groups together with the finding that both groups demonstrated a reduction in carbohydrate intake at follow up compared with baseline fits with the previously reported findings of a positive association of Bacteroides abundance with carbohydrate intake (Fava et al., 2013, Russell et al., 2011b). Bacteroides spp. have diverse saccharolytic capabilities (Flint et al., 2012) and differences in intake of major carbohydrate sources between groups (e.g. rice, oats in low FODMAP vs wheat in sham) may have led to the different magnitude of response in Bacteroides between groups.

There was no difference in total bacteria or *F. prausnitzii* between the low FODMAP diet group and the sham diet group which supports previous work comparing low FODMAP advice to habitual diet (Staudacher et al., 2012) but is in contrast with a feeding study suggesting FODMAP restriction leads to a reduction in *F. prausnitzii* compared with habitual diet (Halmos et al., 2015). This discrepancy is likely due to the differences in study design. The feeding study reduced FODMAP intake dramatically, which is more likely to impart extensive and widespread alterations in the microbiota compared with the current RCT in which free living patients consume self-selected foods and fluids based on dietary advice, as occurs in clinical practice. The preservation of *F. prausnitzii* in response to low FODMAP dietary advice in this RCT is reassuring given its role as a 'keystone species', its major contribution to commensal butyrate production and the proposal that it could be a biomarker of intestinal health in adults (Miquel et al., 2013).

Other important organisms that may have relevance for health were unaltered by low FODMAP dietary advice. These include the mucin degrader *A. muciniphila* and the butyrate producer *R. bromii* which have been shown to be diet-sensitive, specifically to polyphenols and resistant starch, respectively (Anhe et al., 2015, Walker et al., 2011). Therefore, it could be proposed that a significant reduction in FODMAP intake, which reduces prebiotic intake, leads to a reduced abundance of Bifidobacteria and Roseburia spp., but other diet-sensitive microorganisms appeared to be preserved due to sufficient intake of other dietary components (e.g. resistant starch, polyphenols), though intakes of these components were not measured here.

6.3.2 Stool SCFA and pH

This is the first report that a low FODMAP diet leads to lower stool acetate concentration compared with controls. This alteration occurred in the absence of significant differences in total SCFA or other individual SCFA, although there was a trend for a lower total SCFA concentration in the low FODMAP diet group compared with sham ($p=0.061$). Two studies have examined the effect of the low FODMAP diet on stool SCFA and found no impact of the low FODMAP diet, both were smaller studies that may not have been sufficiently powered to detect differences (Staudacher et al., 2012, Halmos et al., 2015) and one also pooled samples from healthy subjects and IBS which may have masked differences in patients (Halmos et al., 2015).

The reason for this alteration in stool acetate concentration is unclear and likely multi-dimensional. The most plausible explanation is a reduction in the abundance of the acetate-producing Bifidobacteria. It could also be due to reduced abundance of other microorganisms that generate acetate (*Ruminococcus* spp. and *Clostridium* spp.), increased colonic absorption of acetate or prolonged colonic transit time. Butyrate-producers (especially *F. prausnizii* and Roseburia spp. & *E. rectale*) also utilise acetate in cross feeding reactions (Duncan et al., 2004), and therefore increased utilisation through this pathway may have also contributed.

Although novel in terms of a low FODMAP diet, the reduced stool acetate concentration is not a new finding when considering studies that alter total dietary carbohydrate intake. For example, carbohydrate restriction leads to reduction in stool acetate in obese individuals over 3-8 weeks (Brinkworth et al., 2009, Russell et al., 2011b, Salonen et al., 2014) and a similar effect has been demonstrated in healthy individuals after five days of a fibre-free high protein diet (David et al., 2014). Furthermore, individuals following agrarian diets or plant-based diets have higher stool acetate concentration compared with controls (De Filippo et al., 2010, De Filippis et al., 2015).

The effect of VSL#3 probiotic on stool SCFA in IBS has never been examined, and there is a lack of data regarding the *in vivo* effect of other Bifidobacteria-containing probiotics on stool SCFA concentration in IBS. Intriguingly, low FODMAP dietary advice with probiotic co-administration (low FODMAP diet + probiotic) in this RCT appeared to somewhat ameliorate the reduction in total SCFA, butyrate, acetate and propionate (Appendix 9.27), although this was not statistically significant. This is also interesting considering that there were no independent effects of VSL#3 on stool SCFA concentrations compared with placebo despite its bifidogenic effect. It is difficult to explain the reason for the absence of a higher stool SCFA concentration in response to VSL#3, but it may be due to shifts in SCFA pathways in the colon at times of greater SCFA availability, such as increased colonic absorption.

It is unknown whether a lower stool acetate concentration has any effect on GI function. Colonic SCFA absorption stimulates sodium-dependent water absorption and therefore it may have led to altered water availability in the colon and possibly altered stool consistency. Furthermore, whether acetate concentration *per se* is specifically associated with other parameters of colonic or overall health in IBS is unknown. It is reassuring that butyrate, known for its protective effect on the colonic mucosa, did not reduce significantly in response to low

FODMAP dietary advice. This may be explained by an unaltered abundance of predominant butyrate producers from Clostridium Cluster XIVa, such as *F. prausnitzii* (Benus et al., 2010), despite a reduction in Roseburia spp. which is positively correlated with stool butyrate concentration (Duncan et al., 2007). The numerous important effects of butyrate in the colon are recognised and well described, but the relevance of a change in stool acetate is less clear. Stool concentration in cancer patients is lower versus controls and it has been demonstrated to induce apoptosis of cancer cells *in vitro*, although less so than butyrate (Topping and Clifton, 2001).

Stool pH in this RCT was unaltered in response to low FODMAP dietary advice or the probiotic intervention. This is largely unsurprising as stool pH is influenced by concentrations of multiple metabolites, not just byproducts of carbohydrate fermentation. For example, protein fermentation by-products (phenols, amines and ammonia) and organic acid by-products of hexose and pentose metabolism (lactate and succinate) also contribute to pH of the lumen (Cummings and Macfarlane, 1991). Furthermore, a majority of carbohydrate fermentation and SCFA production occurs in the caecum, which are then absorbed in the distal colon (Cummings and Macfarlane, 1991), meaning stool pH is limited as a marker for colonic pH.

One study has reported increased stool pH after low FODMAP feeding compared with habitual diet in healthy individuals and IBS patients (Halmos et al., 2015). Stool collection, handling and storage was conducted by participants over a period of five days which may have led to variability and potentially suboptimal sample handling, although this is more likely to have led to increased fermentation and artificially reduced pH of samples, and is likely to have occurred across all samples. Another explanation might be that the stringent FODMAP restriction compared with this RCT led to reduced SCFA production and therefore a higher pH, however no differences were evident in SCFA concentration compared with habitual diet. Stool pH across all groups and timepoints in that study was higher than in this RCT and compared with previously reported data from healthy individuals (Fallingborg et al., 1989), and therefore methodological differences between studies may have contributed to the variability in findings.

Further work is required to characterise the changes in fermentation dynamics in the colon in response to the low FODMAP diet and whether alterations in microbiota by-products, such as SCFA, and their subsequent impact on luminal pH have an impact on GI health.

6.4 Strengths and limitations

There are a number of strengths of this RCT in relation to the findings presented here. Firstly, the application of the intervention through advice from a specialist dietitian and the duration of the intervention closely reflected clinical practice. Therefore the observed effects on the microbiota may closely represent expected outcomes in clinical practice, in contrast to feeding studies where dietary intake is more tightly controlled. A robust technique was used to characterise the microbiota in this RCT, and aspects of microbiota functionality was measured through evaluation of stool SCFA and pH.

There are also some limitations of the current RCT with relation to the evaluation of the microbiota. Firstly, the use of the sham diet in this study, although imperative for placebo-controlled comparison of symptom response, may have had a bearing on comparisons between groups for the microbiota outcomes. The sham diet required a change to food intake which likely had at least a subtle impact on the GI microbiota in some patients. A diet that maintained habitual intake would have been the optimal comparison for evaluation of microbiota response to the low FODMAP diet, however this could not have been classified as a placebo control and would impact on conclusions regarding the clinical effectiveness reported in Chapter 4.

GI microbiota composition was evaluated using qPCR, which is a relatively rapid technique, and an accurate culture independent method for quantifying microbiota. However, it is limited by the number of assays that are practically feasible to include in the experiment. Therefore, there may have been alterations in abundances of other genera and species that were not evident with the primers used here. Furthermore, measures of diversity, that are possible with metagenomic sequencing but not with qPCR, were not evaluated here. This requires evaluation, as microbiota diversity is increasingly recognised as an important marker for health (Lozupone et al., 2012).

Another limitation relates to the measurement of fermentation byproducts. SCFA concentration is likely associated with stool volume (Cummings and Macfarlane, 1991), and stool volume was not measured in this study. Therefore, a change in stool volume in response to the interventions may have biased these outcomes. Furthermore, SCFA measured in stool is the net product of the dynamic process of both SCFA production and absorption, the latter of which is affected by colonic transit time (Cummings and Macfarlane, 1991). Precise

measurement of *in vivo* local SCFA production and luminal pH throughout the colon is difficult due to the inaccessibility of the caecum and proximal colon where the majority carbohydrate fermentation takes place (Cummings and Macfarlane, 1991).

Estimation of local fermentation rates throughout the colon would be possible using a wireless motility capsule device to measure pH. Measurement of transit time is also possible with this device, which is associated with alteration in microbiota composition and function (Kashyap et al., 2013). There are also numerous methods of estimating protein fermentation (Yao 2015), which, if maintained throughout a low FODMAP diet, might allow more precise judgements about the contribution of carbohydrate fermentation alteration to stool pH. Metabolomic techniques also present a possible solution for measuring the functional response of the microbiota to diet.

Another shortcoming of this RCT relates to the explanation for the diet-induced alterations in the microbiota. Although the aim of the low FODMAP diet was to simply restrict fermentable carbohydrates, there may have been unmeasured alterations in dietary diversity or other dietary components that contribute significantly to colonic fermentation (e.g. resistant starch) and/or to microbiota composition (e.g. polyphenols, pectin). This problem of collinearity is, however, almost unavoidable in studies that manipulate whole diets and especially in dietary advice studies where patients have freedom of dietary choice for an extended period of time. Therefore, without a complete composition analysis of all foods and fluids consumed, which is not feasible in a dietary advice study, the dietary influences responsible for the change in the microbiota in the low FODMAP group cannot be confirmed.

6.5 Significance of the findings

This is the largest evaluation of the effect of low FODMAP dietary advice on the GI microbiota in patients with IBS, and the first to investigate whether the diet-induced effect on the microbiota can be prevented by an adjunct therapy. The findings confirm the Bifidobacteria-lowering effect of the low FODMAP diet, which has been shown previously in smaller RCTs (Staudacher et al., 2012, Halmos et al., 2015), and is proposed to be largely due to a reduced intake of prebiotic fructans and GOS. There was also a bifidogenic effect of VSL#3, as demonstrated previously (Brigidi et al., 2000, Brigidi et al., 2001, Brigidi et al., 2003). Novel findings are also presented, including evidence that the low FODMAP diet reduces Bifidobacteria species (*B. longum and B. adolescentis*) and Roseburia spp. & *E. rectale*, as well

as stool acetate concentration. Additionally, the co-administration of probiotic ameliorated the diet-induced effect on the Bifidobacteria. The implications of these findings for clinical practice are presented in Chapter 7.

These findings were evident in a homogenous cohort of patients with IBS-D, IBS-M and IBS-U whose habitual dietary intake is broadly similar to the general UK population. Therefore it is assumed the findings here are representative of the effect of the low FODMAP diet in the majority of patients with IBS who comply with low FODMAP dietary advice. Whether the findings can be applied to patients who may have vastly different microbiota composition (e.g. patients who currently take a probiotic, have comorbidity, are obese, or the young or elderly) or who are less compliant with dietary advice compared with patients in this RCT, is unknown.

6.6 Conclusion

This RCT confirms that low FODMAP dietary advice leads to alteration in the abundance of a number of bacterial groups, and on their metabolic byproducts. Probiotic co-administration ameliorates the diet-induced effect on Bifidobacteria abundance, which may be important due to its established benefits on host function (Lee and O'Sullivan, 2010), and that Bifidobacteria is associated with clinical symptoms in IBS. It remains to be determined whether the disturbance induced by the low FODMAP diet is extreme enough to lead to persistent alteration over time. Furthermore, the effect of FODMAP reintroduction on the GI microbiota and its byproducts requires evaluation.

7 Final discussion

7.1 Summary of findings

Irritable bowel syndrome is a common GI disorder that has a marked impact on HRQOL (Gralnek et al., 2000). The heterogeneity of the condition and an incomplete understanding of its pathophysiology have contributed to the lack of uniformly successful treatment approaches. Moreover, many patients and their healthcare providers consider diet has a key role in managing symptoms (Hungin et al., 2014, Halpert et al., 2007). This is supported by the growing body of evidence confirming the effectiveness of dietary intervention in managing IBS symptoms, although it is acknowledged that gold standard placebo-controlled dietary intervention RCTs are difficult to conduct.

The aim of Chapter 3 was to describe the design, development and evaluation of a novel sham diet for use as a placebo comparator in a 2x2 factorial design dietary advice RCT investigating the effect of low FODMAP dietary advice and probiotic supplementation in patients with IBS. The main aims of the sham diet were to alter carbohydrate sources but maintain FODMAP and nutrient intake, whilst being feasible to follow and convincing as an exclusion diet. Outcomes of a pilot study and an interim analysis of FODMAP and nutrient intake from the RCT confirmed the suitability of the sham diet for use as a placebo control in this study.

The aim of Chapter 4 was to report the results for the clinical outcomes of the low FODMAP dietary advice RCT. Convincing evidence was presented that low FODMP dietary advice has a beneficial impact on symptom and HRQOL scores compared with placebo sham dietary advice. Low FODMAP dietary advice was effective even though it was provided without a description of the underlying physiological effects of FODMAPs in the GI tract. The acceptability data presented in this chapter, however, indicated a large proportion of patients reported difficulty with practical aspects of the low FODMAP diet. Acceptability may be higher in clinical practice where extra supportive material and time is dedicated to the education process.

Data from this RCT, also presented in Chapter 4, suggests that the effect of VSL#3 on GI symptoms is equivocal, although it is acknowledged there was insufficient power to detect differences for probiotic over placebo. There was no interaction between the two interventions (low FODMAP diet and probiotic), and therefore statistically speaking, the effect of low FODMAP dietary advice and VSL#3 on GI symptoms is considered additive. On subgroup analysis of the intervention combination groups for IBS-SSS outcomes there were no difference

when low FODMAP dietary advice was provided alone compared with when it was co-administered with the probiotic (i.e. low FODMAP diet + placebo vs low FODMAP diet + probiotic), and therefore it remains to be confirmed whether low FODMAP dietary advice with probiotic is more effective for improving symptoms than low FODMAP dietary advice alone.

Clearly, the clinical response to low FODMAP dietary advice and to probiotic based on adequate relief in this RCT is in stark contrast to outcomes from the IBS-SSS, GSRS and HRQOL instruments. Recently, the same discrepancy was noted in 75 patients with IBS randomised to either a low FODMAP-low gluten diet or a low FODMAP-normal gluten diet (Piacentino et al., 2015). When compared with controls consuming a normal diet, there was no difference between groups using a dichotomous endpoint, but clear differences were evident when global symptoms were rated using a VAS. The authors concluded that a VAS better discriminated treatment outcome compared with a dichotomous response question.

This incongruous relationship was explored further by grouping patients from the current RCT according to the presence or absence of adequate relief (**Table 7.1**). Clear differences in IBS-SSS total score and subscores were evident between the groups. This confirms that a positive response to the adequate relief question in this RCT was generally accompanied by a lower mean IBS-SSS score.

Table 7.1 IBS-SSS scores at follow up grouped by adequate relief response for patients that completed a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=95)

	Adequate relief (n=48)	Absence of adequate relief (n=47)	p[#]
IBS-SSS score (pts)	140.5 (81.6)	249.6 (76.3)	**<0.001**
Severity of pain	26.3 (27.4)	46.0 (27.7)	**<0.001**
Days of pain (days)	23.5 (20.7)	47.1 (20.1)	**<0.001**
Distension severity	24.5 (21.3)	44.4 (25.6)	**<0.001**
Satisfaction with bowels	28.8 (16.8)	55.5 (14.8)	**<0.001**
Affecting life	37.4 (19.9)	56.7 (17.3)	**<0.001**
Change in IBS-SSS (pts)	-124.0 (87.0)	-50.7 (72.2)	**<0.001**
Achieved IBS-SSS MCID n (%)	39 (81)	20 (43)	**<0.001**

All units are (mm) unless stated. Values are mean (SD). [#] Mann-Whitney U tests for continuous variables and Chi-squared test for categorical variable

When patients were grouped according to baseline IBS-SSS severity, however, the nature of the discrepancy between adequate relief and IBS-SSS outcomes became clear (**Figure 7.1**). Patients with severe IBS symptoms at baseline (according to the IBS-SSS) demonstrated the greatest reduction in IBS-SSS score (mean -116 points) compared with those with moderate (-76 points) and mild symptoms (-16 points). However, those with severe symptoms reported adequate relief the least (34%) compared with those with moderate (62%) or mild symptoms (40%). This suggests that for many patients with severe symptoms who experienced a large reduction in IBS-SSS score, of even more than 2-fold the MCID, this level of clinical response is still insufficient for them to report 'adequate relief'.

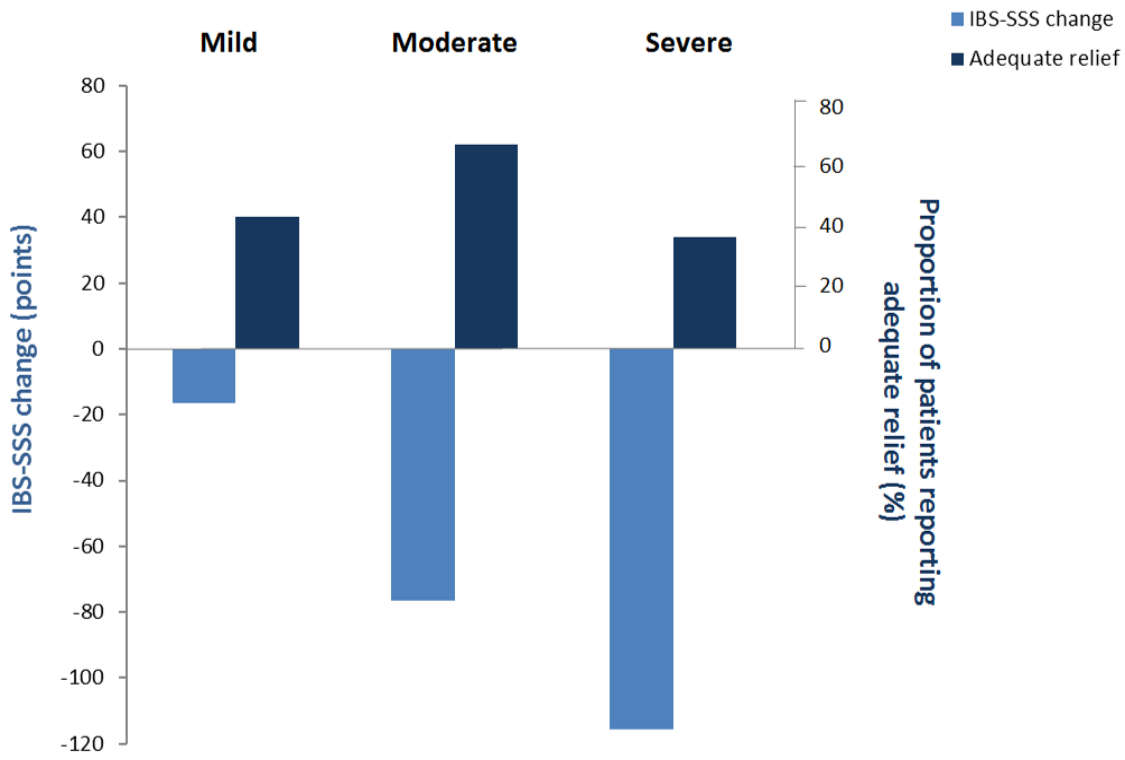


Figure 7.1 Change in IBS-SSS score and proportion of patients reporting adequate relief at follow up for patients with mild, moderate or severe symptoms at baseline. Patients included completed a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=95; severity categories based on IBS-SSS: mild 75-174 pts, moderate 175-300 pts, severe 301-500 pts)

A similar pattern of findings has been reported in a large cohort of primary and secondary care patients with IBS treated with standard medical care. A lower proportion of patients with severe IBS symptoms reported adequate relief after the six month intervention compared with those with mild or moderate symptoms, whereas patients with severe symptoms exhibited the greatest change in IBS-SSS score at 6 months (Whitehead et al., 2006). In contrast, this same discrepancy was not detected in a large pooled analysis of 12 RCTs (Spiegel et al., 2009), however in this study adequate relief response was compared with a 50% improvement endpoint, a different endpoint to IBS-SSS change, which was used in the current exploratory analysis. Overall, it can be concluded that clinical response based on the adequate relief dichotomous endpoint may underestimate the effectiveness of an intervention, particularly in patients with severe symptoms. Indeed, when considering these data and the disadvantages of the adequate relief question that have already been discussed (Section 2.5 and Section 4.7.1), use of this dichotomous outcome as a primary endpoint to discriminate clinical responders from non-responders is deemed questionable.

The aim of Chapter 5 was to report the results of low FODMAP dietary advice on nutrient intake of patients in the RCT. The results supported data from the interim analysis presented in Chapter 3, demonstrating that FODMAP intake remained stable in the sham diet group and reduced in the low FODMAP diet group. There were some minor alterations to nutrient intake in the sham diet group compared with the low FODMAP diet group but this was for micronutrients (e.g. potassium, magnesium) that should not have confounded microbiology or symptom outcomes. NSP intake in the low FODMAP diet group did not change, confirming that altered NSP intake was not responsible for the improvement in GI symptoms in response to low FODMAP dietary advice. Furthermore, nutrient intake in patients prior to and following the low FODMAP diet was broadly similar to the UK population. The finding that the proportion of patients that achieved the RNI for calcium was lower after low FODMAP dietary advice is in line with previous work (Staudacher et al., 2012) and highlights the importance of patients consuming low lactose, high calcium alternative foods while on the low FODMAP diet.

Over two thirds of the sham diet group (71%) guessed their allocation to the sham diet, which may have been due to a lack of symptom improvement in this group. The symptom response rate, or placebo effect, in the sham diet group was 38% for adequate relief and 42% for those achieving the MCID for IBS-SSS, figures in line with overall placebo response rates reported for

IBS (20-40%) (Elsenbruch and Enck, 2015), suggesting that suspicion of allocation appeared not to heavily impact on placebo response rate in the sham diet group.

The aim of Chapter 6 was to report the results for the GI microbiota and markers of fermentation from the RCT. qPCR findings showed low FODMAP dietary advice led to a reduction in the abundance of stool Bifidobacteria, which confirms previous data (Staudacher et al., 2012, Halmos et al., 2015), and also demonstrated that the degree of reduction was negatively correlated with baseline concentration. This is the first data to show that there is a reduced abundance of *B. longum, B. adolescentis* and Roseburia spp. & *E. rectale* in response to low FODMAP dietary advice, which was likely due to decreased availability of fermentable carbohydrate substrate for these saccharolytic organisms, and an outcome that may not have previously been demonstrated due to smaller sample sizes. For the first time, it was demonstrated that low FODMAP dietary advice led to reduced stool acetate concentration, a byproduct of carbohydrate fermentation which has been previously shown to reduce in response to decreased carbohydrate intake (David et al., 2014).

It could be argued that the low FODMAP diet-induced effect on the microbiota may have been underestimated in this RCT due to the reduced intake of carbohydrate, starch and NSP in the sham diet group. This may have had an unintended effect on the microbiota in response to the sham diet, thereby masking broader alterations in the microbiota in the low FODMAP diet group that were not evident from the current data. The ideal method to test this would be to compare the microbiota composition of patients following low FODMAP advice with patients consuming habitual diet, although this would not suffice as a placebo control group for investigating symptom endpoints. Considering that the absolute change in carbohydrate and starch intake was not different between the low FODMAP and sham diet groups, it could be argued that microbiota findings here are representative of what occurs in response to FODMAP restriction *per se* and not to an alteration in other dietary components such as total carbohydrate or starch.

Probiotic supplementation with VSL#3 generally led to changes in the microbiota that had been anticipated prior to the RCT (**Figure 2.2**). Enhanced Bifidobacteria abundance was apparent in the probiotic group compared with placebo. Interestingly, there was no difference in Lactobacillus abundance between groups. However, there was a significantly lower

proportion of patients in the probiotic group with Lactobacillus numbers below the detection limit, indicating that probiotic supplementation had led to colonisation in the GI tract.

As was the case for symptom analysis, the lack of an interaction between diet and product interventions for the microbiota analysis suggests that their impact is additive and not synergistic or antagonistic. This effect is evident when Bifidobacteria abundance for combination intervention groups was compared. However, it may be that the response in all patients is not simply additive in nature. As suggested by the inverse correlation between baseline Bifidobacteria and Bifidobacteria change in response to dietary intervention (**Figure 6.1**), there is likely substantial inter-individual variability in the microbiota alteration in response to low FODMAP diet and probiotic co-administration. Sufficiently powered studies are required to clarify the extent and nature of individual microbiota responses to combined low FODMAP diet and microbiota-targeted treatments (e.g. probiotic or prebiotics).

7.2 Predictors of symptom response to low FODMAP dietary advice

Identifying determinants of response to a disease intervention is important for timely treatment of the condition. This is particularly so for the heterogeneous disorder of IBS for which there is an extensive array of medical, cognitive and/or dietary management strategies available.

Adherence is a factor associated with greater symptom response to the low FODMAP diet in patients with IBS (Shepherd and Gibson, 2006). Demographic and clinical variables may also be potential markers of response. For example, one study compared responders (n=19) versus non-responders (n=19) to low FODMAP dietary advice (Bohn et al., 2015) and found a higher proportion of females and older age in responders compared with non-responders (Bohn et al., 2015). In contrast, one small study (n=8) in children failed to demonstrate any differences in gender, BMI, ethnicity or clinical parameters (14-hour hydrogen or methane production, pain frequency, stool frequency or transit time) between responders and non-responders, although this is likely to have been inadequately powered to detect such differences (Chumpitazi et al., 2014).

Dietary composition may be an important baseline factor in predicting response to the low FODMAP diet. Overall nutrient composition was not different between responders and non-

responders in one study (Chumpitazi et al., 2015), however a lower baseline dietary intake of FODMAPs was identified in responders compared with non-responders in a recent study in adults (Bohn et al., 2015). This might suggest that patients who previously self-restricted high FODMAP foods (perhaps due to a perception that they provoke symptoms) are more likely to achieve benefit (Bohn et al., 2015). Overall, however, there is limited consistent evidence that suggests demographic, clinical or dietary factors predict response to the low FODMAP diet in patients with IBS.

There are several limitations of the studies investigating predictors of response to low FODMAP restriction. Firstly, limitations relate to aspects of study design. The crossover nature of studies (Halmos et al., 2015, Chumpitazi et al., 2015) are potentially problematic, as they hold risk of carryover effects, particularly where interventions are separated by less than a week (Chumpitazi et al., 2015). This is particularly important for symptom endpoints, as carryover in the second arm could influence whether 'response' was achieved or not. Also, two of the three studies were feeding studies (Halmos et al., 2015, Chumpitazi et al., 2015) which may not have been representative of a low FODMAP diet that is achievable in the clinical setting and therefore the findings may not reflect outcomes in patients following a self-selected low FODMAP diet.

Another limitation of the studies relates to methodological aspects. Firstly, two of the three studies were in children (Chumpitazi et al., 2015, Chumpitazi et al., 2014) and whether any potential predictors of response in children are also applicable to adults is unknown. Furthermore, the duration of the dietary intervention (two days to three weeks) would have had a major impact on the likelihood of response, and indeed an unusually low response rate was reported in two of the studies (response rates 8/33 and 4/8) where the duration of the dietary intervention was two days (Chumpitazi et al., 2015) and one week, respectively (Chumpitazi et al., 2014). Finally, and importantly, the definition of a 'responder' to the low FODMAP diet varies between studies. Responder definitions have included ≥50% reduced pain frequency in children (Chumpitazi et al., 2014, Chumpitazi et al., 2015), a score lower compared with control intervention (> 20 mm below those receiving a control diet) (Halmos et al., 2015), or reaching the MCID for the IBS-SSS (Bohn et al., 2015). Therefore, a parallel study in a large cohort using appropriate response criteria is required to clarify whether response is determined by baseline variables.

Multiple logistic regression analysis was undertaken to predict response in the low FODMAP diet group for this RCT based on demographic, clinical severity and dietary variables that previous studies and physiological plausibility indicate may be important in determining response (**Table 7.2**). Controlling for the other variables, the only variable that predicted achieving the MCID for IBS-SSS (50-point reduction in score) was change in FODMAP intake. For every one gram reduction in FODMAP intake at follow up compared with baseline, there was a 1.14 odds (95% CI 1.01, 1.29) of achieving the MCID for IBS-SSS score. Variables that did not predict response were age (p=0.390), duration of symptoms (p=0.327), baseline IBS severity (p=0.365) and baseline FODMAP intake (p=0.390).

Table 7.2 Multiple logistic regression analysis assessing predictors for meeting the MCID for IBS-SSS score in patients allocated to the low FODMAP diet group (n=51) in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (responders n=37)

Variable	OR	95% CI	p
Age (yrs)	1.03	0.97, 1.09	0.390
Duration of symptoms (mths)	1.00	0.99, 1.00	0.327
IBS-SSS at baseline (pts)	1.00	0.99, 1.01	0.365
Total FODMAP intake at baseline(g)	0.95	0.85, 1.07	0.390
Change in FODMAP intake (g)	1.14	1.01, 1.29	**0.033**

OR, odds ratio; CI, confidence interval

This is the largest evaluation of predictors of response to low FODMAP dietary advice (37 responders) using an established response definition based on the validated IBS-SSS, as used in previous responder analysis (Bohn et al., 2015). The results suggest that certain demographic and clinical factors are not important determinants of response to low FODMAP dietary advice. Furthermore, patients with a low baseline FODMAP intake will have a likelihood of response to low FODMAP dietary advice that is equivalent to patients with a high baseline intake. This is in contrast to previous work (Bohn et al., 2015), which may be due to a much larger sample in the current analysis (37/51 responders vs 19/38 responders in previous study). That change in FODMAP intake is predictive of response is somewhat expected, and reassuring, and provides further evidence that it is FODMAP restriction that is responsible for the effectiveness of the low FODMAP diet, rather than other unintended unmeasured dietary alteration.

7.3 Implications for clinical practice

The results of this RCT provide compelling evidence that low FODMAP dietary advice is effective for improving GI symptoms and HRQOL in patients with IBS (57% of patients based on the adequate relief question and 73% based on the MCID for IBS-SSS). It might be interpreted that low FODMAP dietary advice with co-administered VSL#3 is superior to low FODMAP dietary advice alone when considering its effect on Bifidobacteria abundance. However, the microbiota is a resilient ecosystem, and has ability to recover after perturbation (Relman, 2012), and it remains to be determined whether the disturbance induced by the low FODMAP diet alone is extreme enough to lead to persistent alteration over time. It is also vital that the effect of graded FODMAP reintroduction on the GI microbiota and its byproducts is investigated, not only as this is part of routine clinical practice, but also as there is considerable cost associated with probiotic co-administration (currently £90/patient for a 4-week course of VSL#3). In summary, the implications for clinical practice from this work are as follows:

1) Low FODMAP dietary advice should remain a primary dietary strategy for managing IBS symptoms if first line dietary and lifestyle intervention is ineffective (McKenzie et al., 2012). It is recommended that current guidelines advising the consideration of low FODMAP dietary advice in patients with IBS and bloating (McKenzie et al., 2012) be revised to include all patients with IBS-D, IBS-M or IBS-U.
2) The effect of VSL#3 alone for specific GI symptoms is equivocal, despite its impact on increasing stool Bifidobacteria abundance, and there is insufficient evidence to recommend its routine use in patients with IBS-D, IBS-M and IBS-U.
3) In terms of symptom benefit, there is currently insufficient evidence to recommend that low FODMAP diet-VSL#3 combination therapy be used in routine practice in preference to the low FODMAP diet alone.
4) Although the long term effects on the microbiota were not investigated here, the acute effects of the low FODMAP diet on the microbiota suggest that reintroduction of FODMAPs to tolerance should be undertaken, with specific emphasis on prebiotic fructans and GOS.
5) Low FODMAP dietary advice should be provided by a registered dietitian. Advice should be individualised, and in view of the impact on calcium intake, emphasis should be placed on inclusion of low lactose calcium-rich alternatives, or a calcium

supplement should be considered if intake of sufficient dietary calcium is not achievable.

7.4 Recommendations for future research

A number of key areas for further research emerge based on the current findings. The RCT evidence for the clinical response to the low FODMAP diet is growing, particularly for patients in secondary care, however its effect on clinical outcome and dietary intakes in dietitian-led, general practitioner-led and self-taught patients in the community setting requires further study. The effect of low FODMAP dietary advice in IBS-C is also unclear. The use of a sham diet, such as that developed here, is recommended as a feasible and effective placebo comparator for placebo-controlled RCTs investigating the impact of the low FODMAP dietary on symptom response.

With the growing evidence of the role of the GI microbiota in health and disease, more research is required evaluating the effect of the low FODMAP diet on the composition and community structure of the GI microbiota. The timepoint at which the microbiota alterations occur, the impact of long term FODMAP restriction and the ramifications of these changes (if any) on colonic health require investigation. Whether the mucosal microbiota is altered, particularly as mucosal abnormalities are already evident in IBS compared with healthy controls (Section 1.1.7.4), requires investigation. It is vital that prospective studies addressing these research questions attempt to control for confounding factors that were not measured here, such as stress (Bailey et al., 2011) and GI transit (Kashyap et al., 2013), and emerging dietary modulators of the microbiota (e.g. polyphenols), although it is acknowledged that the latter is more feasible in feeding studies than dietary advice studies.

It is understood that there are profound inter-individual differences in the microbiota response to dietary change (Duncan et al., 2007), and this has not been evaluated in patients following a low FODMAP diet. An appreciation of the specificity of the effect on the microbiota between individuals, and whether this is in fact predictive of symptom response may help to form the basis of personalised dietary interventions in the future.

In addition to the host-specific factors that might determine the nature of the diet-induced response of the microbiota, the composition of the low FODMAP diet itself is probably

important. The individual fermentable carbohydrates restricted as part of a low FODMAP diet differ in digestibility and prebiotic potential and therefore each FODMAP probably has unique influences on microbiota composition. It is already known, for example, that GOS are more selectively bifidogenic than fructans, which have broader effects on the microbiota ecosystem (Bindels et al., 2015). The independent and combined effects of FODMAPs on the microbiota require further study and this may modify future application of the diet, particularly in patients with mild symptoms who do not require complete restriction and for which the impact on the microbiota should be minimised.

It is important that future research on the microbiota in this area is not purely focused on the low FODMAP diet-driven changes in its composition, but also on the effect of its metabolism and function. This is particularly important as functionality of the microbiota might be more strongly influenced by dietary change than the composition of the microbiota itself (Salonen et al., 2014)**.** The measurement of bacterial metabolites known to be important in colonic health (e.g. SCFA, phenolic compounds) and disease (e.g. *N*-nitroso compounds, ammonia), some of which have previously been associated with carbohydrate intake (Russell et al., 2011b), will be important in the evaluation of whether the removal of fermentable substrates is potentially damaging to long term health. Indeed, these insights may lead to the development of targeted interventions that improve IBS symptoms but optimise colonic health (e.g. low FODMAP diet combined with added fibre/prebiotic/probiotic).

Finally, a number of recommendations arise in relation to measuring symptom response in IBS. Consideration of the primary clinical endpoint in this work (adequate relief of symptoms) in isolation would have led to a vastly different interpretation of the clinical effect of the interventions. There are obvious limitations of the use of this dichotomous endpoint alone as discussed in Section 2.5.1, Section 4.7.1 and Section 7.1. It is recommended that where future studies use dichotomous endpoints that the following should be applied: 1) combination endpoints e.g. adequate relief question and proportion of patients meeting the MCID for IBS-SSS; or 2) the US Food and Drug Administration subtype-specific recommendations, although these are limited to IBS-C and IBS-D (FDA, 2012).

7.5 Conclusion of this doctoral thesis

The low FODMAP diet is becoming an increasingly widespread approach for the management of GI symptoms in patients with IBS. This thesis has presented evidence from a large blinded placebo-controlled RCT that the low FODMAP diet has beneficial clinical impact in up to 73% of patients with IBS-D, IBS-M and IBS-U, and specifically for symptoms such as bloating, borborygmi, flatulence and dissatisfaction with bowel habit (specifically urgency and sensation of incomplete evacuation), which also translates into improved HRQOL outcomes. Dietary intake is not compromised in patients following low FODMAP dietary advice, although this study confirms calcium is an at-risk nutrient that requires emphasis in the clinical consultation. The impact of a 4-week low FODMAP diet on the GI microbiota can be ameliorated by co-administration of a probiotic, and this is potentially advantageous in a condition characterised by dysbiosis, although the impact of FODMAP reintroduction requires evaluation before combined low FODMAP diet-probiotic therapy is recommended as routine practice.

8 References

Agostini, S., Goubern, M., Tondereau, V., Salvador-Cartier, C., Bezirard, V., Leveque, M., Keranen, H., Theodorou, V., Bourdu-Naturel, S., Goupil-Feuillerat, N., Legrain-Raspaud, S. & Eutamene, H. 2012. A marketed fermented dairy product containing Bifidobacterium lactis CNCM I-2494 suppresses gut hypersensitivity and colonic barrier disruption induced by acute stress in rats. *Neurogastroenterol Motil,* 24, 376-e172.

Ahn, J. Y., Lee, K. H., Choi, C. H., Kim, J. W., Lee, H. W., Kim, M. K., Kwon, G. Y., Han, S., Kim, S. E., Kim, S. M. & Chang, S. K. 2014. Colonic mucosal immune activity in irritable bowel syndrome: comparison with healthy controls and patients with ulcerative colitis. *Dig Dis Sci,* 59, 1001-11.

Anhe, F. F., Roy, D., Pilon, G., Dudonne, S., Matamoros, S., Varin, T. V., Garofalo, C., Moine, Q., Desjardins, Y., Levy, E. & Marette, A. 2015. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut,* 64, 872-83.

Ariefdjohan, M. W., Savaiano, D. A. & Nakatsu, C. H. 2010. Comparison of DNA extraction kits for PCR-DGGE analysis of human intestinal microbial communities from fecal specimens. *Nutr J,* 9, 23.

Arizona State University. 2013. Figure series of PCR steps. Retrieved from: http://openwetware.org/wiki/BME100_f2013:W1200_Group7_L4.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T*., et al.* 2011. Enterotypes of the human gut microbiome. *Nature,* 473, 174-80.

Atkinson, W., Sheldon, T. A., Shaath, N. & Whorwell, P. J. 2004. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut,* 53, 1459-64.

Austin, G. L., Dalton, C. B., Hu, Y., Morris, C. B., Hankins, J., Weinland, S. R., Westman, E. C., Yancy, W. S. & Drossman, D. A. 2009. A very low-carbohydrate diet improves symptoms and quality of life in diarrhea-predominant irritable bowel syndrome. *Clin Gastroenterol Hepatol,* 7, 706-708 e1.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. & Gordon, J. I. 2005. Host-bacterial mutualism in the human intestine. *Science,* 307, 1915-20.

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G. & Lyte, M. 2011. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun,* 25, 397-407.

Balsari, A., Ceccarelli, A., Dubini, F., Fesce, E. & Poli, G. 1982. The fecal microbial population in the irritable bowel syndrome. *Microbiologica,* 5, 185-94.

Barbara, G., Stanghellini, V., Brandi, G., Cremon, C., Di Nardo, G., De Giorgio, R. & Corinaldesi, R. 2005. Interactions between commensal bacteria and gut sensorimotor function in health and disease. *Am J Gastroenterol,* 100, 2560-8.

Barbara, G., Stanghellini, V., De Giorgio, R., Cremon, C., Cottrell, G. S., Santini, D., Pasquinelli, G., Morselli-Labate, A. M., Grady, E. F., Bunnett, N. W., Collins, S. M. & Corinaldesi, R. 2004. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology,* 126, 693-702.

Barnard, N. D., Scialli, A. R., Turner-McGrievy, G. & Lanou, A. J. 2004. Acceptability of a low-fat vegan diet compares favorably to a step II diet in a randomized, controlled trial. *J Cardiopulm Rehabil,* 24, 229-35.

Barrett, J. S., Gearry, R. B., Muir, J. G., Irving, P. M., Rose, R., Haines, M., Shepherd, S. J. & Gibson, P. R. 2010. Dietary poorly absorbed, short-chain carbohydrates increase

delivery of water and fermentable substrates to the proximal colon. *Aliment Pharmacol Ther,* 31, 874-882.

Barrett, J. S. & Gibson, P. R. 2010. Development and validation of a comprehensive semi-quantitative food frequency questionnaire that includes FODMAP intake and glycemic index. *J Am Diet Assoc,* 110, 1469-76.

Barrett, J. S., Irving, P. M., Shepherd, S. J., Muir, J. G. & Gibson, P. R. 2009. Comparison of the prevalence of fructose and lactose malabsorption across chronic intestinal disorders. *Aliment Pharmacol Ther,* 30, 165-74.

Bartosch, S., Fite, A., Macfarlane, G. T. & McMurdo, M. E. 2004. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl Environ Microbiol,* 70, 3575-81.

Bate, J. P., Irving, P. M., Barrett, J. S. & Gibson, P. R. 2010. Benefits of breath hydrogen testing after lactulose administration in analysing carbohydrate malabsorption. *Eur J Gastroenterol Hepatol,* 22, 318-26.

Bazzocchi, G., Gionchetti, P., Almerigi, P. F., Amadini, C. & Campieri, M. 2002. Intestinal microflora and oral bacteriotherapy in irritable bowel syndrome. *Dig Liver Dis,* 34 Suppl 2, S48-53.

Behbehani, M. M. 1995. Functional characteristics of the midbrain periaqueductal gray. *Prog Neurobiol,* 46, 575-605.

Bellini, M., Bove, A., Sormani, M. P., Battaglia, E., Bocchini, R., Alduini, P., Bassotti, G., Bruzzi, P. & Pucciani, F. 2010. The daily diary and the questionnaire are not equivalent for the evaluation of bowel habits. *Dig Liver Dis,* 42, 99-102.

Benus, R. F., van der Werf, T. S., Welling, G. W., Judd, P. A., Taylor, M. A., Harmsen, H. J. & Whelan, K. 2010. Association between Faecalibacterium prausnitzii and dietary fibre in colonic fermentation in healthy human subjects. *Br J Nutr,* 104, 693-700.

Berkow, S. E., Barnard, N., Eckart, J. & Katcher, H. 2010. Four therapeutic diets: adherence and acceptability. *Can J Diet Pract Res,* 71, 199-204.

Biesiekierski, J. R., Rosella, O., Rose, R., Liels, K., Barrett, J. S., Shepherd, S. J., Gibson, P. R. & Muir, J. G. 2011. Quantification of fructans, galacto-oligosacharides and other short-chain carbohydrates in processed grains and cereals. *J Hum Nutr Diet,* 24, 154-76.

Bijkerk, C. J., de Wit, N. J., Muris, J. W., Jones, R. H., Knottnerus, J. A. & Hoes, A. W. 2003. Outcome measures in irritable bowel syndrome: comparison of psychometric and methodological characteristics. *Am J Gastroenterol,* 98, 122-127.

Bindels, L. B., Delzenne, N. M., Cani, P. D. & Walter, J. 2015. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol,* 12, 303-10.

Bingham, S. A. 1987. The dietary assessment of individuals; methods, accuracy, new techniques and recommendations. *Nutr Abstracts Rev,* 57, 705-42.

Bingham, S. A., Cassidy, A., Cole, T. J., Welch, A., Runswick, S. A., Black, A. E., Thurnham, D., Bates, C., Khaw, K. T., Key, T. J. & et al. 1995. Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *Br J Nutr,* 73, 531-50.

Bingham, S. A., Gill, C., Welch, A., Day, K., Cassidy, A., Khaw, K. T., Sneyd, M. J., Key, T. J., Roe, L. & Day, N. E. 1994. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *Br J Nutr,* 72, 619-43.

Bland, J. M. & Altman, D. G. 2007. Agreement between methods of measurement with multiple observations per individual. *J Biopharm Stat,* 17, 571-82.

Bohn, L., Storsrud, S., Liljebo, T., Collin, L., Lindfors, P., Tornblom, H. & Simren, M. 2015. Diet low in FODMAPs reduces symptoms of irritable bowel syndrome as well as traditional dietary advice: A randomized controlled trial. *Gastroenterology*, 149, 1399-1407.

Bohn, L., Storsrud, S. & Simren, M. 2013. Nutrient intake in patients with irritable bowel syndrome compared with the general population. *Neurogastroenterol Motil,* 25, 23-30.e1.

Bonnema, A. L., Kolberg, L. W., Thomas, W. & Slavin, J. L. 2010. Gastrointestinal tolerance of chicory inulin products. *J Am Diet Assoc,* 110, 865-8.

Born, P., Sekatcheva, M., Rosch, T. & Classen, M. 2006. Carbohydrate malabsorption in clinical routine: a prospective observational study. *Hepatogastroenterology,* 53, 673-7.

Bouhnik, Y., Raskine, L., Champion, K., Andrieux, C., Penven, S., Jackobs, H. & Simoneau, G. 2007. Prolonged administration of low-dose inulin stimulates the growth of bifidobacteria in humans. *Nutrition Research,* 27, 187-193.

Bourdu, S., Dapoigny, M., Chapuy, E., Artigue, F., Vasson, M. P., Dechelotte, P., Bommelaer, G., Eschalier, A. & Ardid, D. 2005. Rectal instillation of butyrate provides a novel clinically relevant model of noninflammatory colonic hypersensitivity in rats. *Gastroenterology,* 128, 1996-2008.

Bradford, K., Shih, W., Videlock, E. J., Presson, A. P., Naliboff, B. D., Mayer, E. A. & Chang, L. 2012. Association between early adverse life events and irritable bowel syndrome. *Clin Gastroenterol Hepatol,* 10, 385-90.e1-3.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., Bienenstock, J. & Cryan, J. F. 2011. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci USA,* 108, 16050-5.

Brigidi, P., Swennen, E., Vitali, B., Rossi, M. & Matteuzzi, D. 2003. PCR detection of Bifidobacterium strains and Streptococcus thermophilus in feces of human subjects after oral bacteriotherapy and yogurt consumption. *Int J Food Microbiol,* 81, 203-9.

Brigidi, P., Vitali, B., Swennen, E., Altomare, L., Rossi, M. & Matteuzzi, D. 2000. Specific detection of bifidobacterium strains in a pharmaceutical probiotic product and in human feces by polymerase chain reaction. *Syst Appl Microbiol,* 23, 391-9.

Brigidi, P., Vitali, B., Swennen, E., Bazzocchi, G. & Matteuzzi, D. 2001. Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. *Res Microbiol,* 152, 735-41.

Brinkworth, G. D., Noakes, M., Clifton, P. M. & Bird, A. R. 2009. Comparative effects of very low-carbohydrate, high-fat and high-carbohydrate, low-fat weight-loss diets on bowel habit and faecal short-chain fatty acids and bacterial populations. *Br J Nutr,* 101, 1493-502.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J. & Wittwer, C. T. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem,* 55, 611-22.

Canavan, C., West, J. & Card, T. 2014. Review article: the economic impact of the irritable bowel syndrome. *Aliment Pharmacol Ther,* 40, 1023-34.

Carlin, A. & Alfirevic, Z. 2008. Physiological changes of pregnancy and monitoring. *Best Pract Res Clin Obstet Gynaecol,* 22, 801-23.

Carroccio, A., Mansueto, P., Morfino, G., D'Alcamo, A., Di Paola, V., Iacono, G., Soresi, M., Scerrino, G., Maresi, E., Gulotta, G., Rini, G. & Bonventre, S. 2013. Oligo-antigenic diet in the treatment of chronic anal fissures. Evidence for a relationship between food hypersensitivity and anal fissures. *Am J Gastroenterol,* 108, 825-32.

Carroll, I. M., Chang, Y. H., Park, J., Sartor, R. B. & Ringel, Y. 2010. Luminal and mucosal-associated intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Gut Pathog,* 2, 19.

Carroll, I. M., Ringel-Kulka, T., Siddle, J. P. & Ringel, Y. 2012. Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol Motil,* 24, 521-e248.

Chang, J. Y., Locke, G. R., 3rd, McNally, M. A., Halder, S. L., Schleck, C. D., Zinsmeister, A. R. & Talley, N. J. 2010. Impact of functional gastrointestinal disorders on survival in the community. *Am J Gastroenterol,* 105, 822-32.

Charbonneau, D., Gibb, R. D. & Quigley, E. M. 2013. Fecal excretion of Bifidobacterium infantis 35624 and changes in fecal microbiota after eight weeks of oral supplementation with encapsulated probiotic.) *Gut Microbes,* 4, 201-11.

Chumpitazi, B. P., Cope, J. L., Hollister, E. B., Tsai, C. M., McMeans, A. R., Luna, R. A., Versalovic, J. & Shulman, R. J. 2015. Randomised clinical trial: gut microbiome biomarkers are associated with clinical response to a low FODMAP diet in children with the irritable bowel syndrome. *Aliment Pharmacol Ther,* 42, 418-27.

Chumpitazi, B. P., Hollister, E. B., Oezguen, N., Tsai, C. M., McMeans, A. R., Luna, R. A., Savidge, T. C., Versalovic, J. & Shulman, R. J. 2014. Gut microbiota influences low fermentable substrate diet efficacy in children with irritable bowel syndrome. *Gut Microbes,* 5, 165-75.

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., Harris, H. M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G. F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D*., et al.* 2012. Gut microbiota composition correlates with diet and health in the elderly. *Nature,* 488, 178-84.

Clarke, S. F., Murphy, E. F., O'Sullivan, O., Lucey, A. J., Humphreys, M., Hogan, A., Hayes, P., O'Reilly, M., Jeffery, I. B., Wood-Martin, R., Kerins, D. M., Quigley, E., Ross, R. P., O'Toole, P. W., Molloy, M. G., Falvey, E*., et al.* 2014. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut,* 63, 1913-20.

Clave, P., Acalovschi, M., Triantafillidis, J. K., Uspensky, Y. P., Kalayci, C., Shee, V. & Tack, J. 2011. Randomised clinical trial: otilonium bromide improves frequency of abdominal pain, severity of distention and time to relapse in patients with irritable bowel syndrome. *Aliment Pharmacol Ther,* 34, 432-42.

Codling, C., O'Mahony, L., Shanahan, F., Quigley, E. M. & Marchesi, J. R. 2010. A molecular analysis of fecal and mucosal bacterial communities in irritable bowel syndrome. *Dig Dis Sci,* 55, 392-7.

Colagiuri, B. 2010. Participant expectancies in double-blind randomized placebo-controlled trials: potential limitations to trial validity. *Clin Trials,* 7, 246-55.

Coletta, M., Di Palma, L., Tomba, C. & Basilisco, G. 2010. Discrepancy between recalled and recorded bowel habits in irritable bowel syndrome. *Aliment Pharmacol Ther,* 32, 282-8.

Collado, M. C., Derrien, M., Isolauri, E., de Vos, W. M. & Salminen, S. 2007. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Appl Environ Microbiol,* 73, 7767-70.

Collins, S. M. 2014. A role for the gut microbiota in IBS. *Nat Rev Gastroenterol Hepatol,* 11, 497-505.

Costabile, A., Klinder, A., Fava, F., Napolitano, A., Fogliano, V., Leonard, C., Gibson, G. R. & Tuohy, K. M. 2008. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. *Br J Nutr,* 99, 110-20.

Crouzet, L., Gaultier, E., Del'Homme, C., Cartier, C., Delmas, E., Dapoigny, M., Fioramonti, J. & Bernalier-Donadille, A. 2013. The hypersensitivity to colonic distension of IBS patients

can be transferred to rats through their fecal microbiota. *Neurogastroenterol Motil,* 25, e272-82.

Crumb-Johnson, R., Smith-Banes, M., Hatcher, L. & Hagan, D. W. 1993. Assessment of differences between compliers and noncompliers in outpatient research diet studies. *J Am Diet Assoc,* 93, 1041-2.

Cummings, J. H. & Macfarlane, G. T. 1991. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol,* 70, 443-59.

Cummings, J. H. & Stephen, A. M. 2007. Carbohydrate terminology and classification. *Eur J Clin Nutr,* 61 Suppl 1, S5-18.

Dai, C., Guandalini, S., Zhao, D. H. & Jiang, M. 2012. Antinociceptive effect of VSL#3 on visceral hypersensitivity in a rat model of irritable bowel syndrome: a possible action through nitric oxide pathway and enhance barrier function. *Mol Cell Biochem,* 362, 43-53.

Dalvit-McPhillips, S. 1984. A dietary approach to bulimia treatment. *Physiol Behav,* 33, 769-75.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J. & Turnbaugh, P. J. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature,* 505, 559-63.

Davis, L. M., Martinez, I., Walter, J., Goin, C. & Hutkins, R. W. 2011. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One,* 6, e25200.

De Filippis, F., Pellegrini, N., Vannini, L., Jeffery, I. B., La Storia, A., Laghi, L., Serrazanetti, D. I., Di Cagno, R., Ferrocino, I., Lazzi, C., Turroni, S., Cocolin, L., Brigidi, P., Neviani, E., Gobbetti, M., O'Toole, P. W.*, et al.* 2015. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut*. Epub ahead of print.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G. & Lionetti, P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA,* 107, 14691-6.

De Palma, G., Nadal, I., Collado, M. C. & Sanz, Y. 2009. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects. *Br.J Nutr,* 102, 1154-1160.

de Roest, R. H., Dobbs, B. R., Chapman, B. A., Batman, B., O'Brien, L. A., Leeper, J. A., Hebblethwaite, C. R. & Gearry, R. B. 2013. The low FODMAP diet improves gastrointestinal symptoms in patients with irritable bowel syndrome: a prospective study. *Int J Clin Pract,* 67, 895-903.

Dear, K. L., Elia, M. & Hunter, J. O. 2005. Do interventions which reduce colonic bacterial fermentation improve symptoms of irritable bowel syndrome? *Dig Dis Sci,* 50, 758-66.

Del Piano, M., Morelli, L., Strozzi, G. P., Allesina, S., Barba, M., Deidda, F., Lorenzini, P., Ballaré, M., Montino, F., Orsello, M., Sartori, M., Garello, E., Carmagnola, S., Pagliarulo, M. & Capurso, L. 2006. Probiotics: from research to consumer. *Dig Liver Dis,* 38 Suppl 2, S248-55.

Department of Health 2004. *Dietary reference values for food energy and nutrients for the United Kingdom,* Norwich, UK, The Stationery Office.

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F. & Dinan, T. G. 2010. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience.* 170, 1179-1188.

Dethlefsen, L., Huse, S., Sogin, M. L. & Relman, D. A. 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol,* 6, e280.

Di Stefano, M., Miceli, E., Gotti, S., Missanelli, A., Mazzocchi, S. & Corazza, G. R. 2007. The effect of oral alpha-galactosidase on intestinal gas production and gas-related symptoms. *Dig Dis Sci,* 52, 78-83.

Didari, T., Mozaffari, S., Nikfar, S. & Abdollahi, M. 2015. Effectiveness of probiotics in irritable bowel syndrome: Updated systematic review with meta-analysis. *World J Gastroenterol,* 21, 3072-84.

Digestive Health Foundation 2006. Irritable bowel syndrome. NSW, Australia, Gastroenterological Society of Australia. Retrieved from: www.gesa.org.au/professional.asp?cid=9&id=125

Doron, S. & Snydman, D. R. 2015. Risk and safety of probiotics. *Clin Infect Dis,* 60 Suppl 2, S129-34.

Dossett, M. L., Davis, R. B., Lembo, A. J. & Yeh, G. Y. 2014. Complementary and alternative medicine use by US adults with gastrointestinal conditions: Results from the 2012 National Health Interview Survey.) *Am J Gastroenterol. 109, 1705-11*.

Drossman, D., Morris, C. B., Hu, Y., Toner, B. B., Diamant, N., Whitehead, W. E., Dalton, C. B., Leserman, J., Patrick, D. L. & Bangdiwala, S. I. 2007. Characterization of health related quality of life (HRQOL) for patients with functional bowel disorder (FBD) and its response to treatment. *Am J Gastroenterol,* 102, 1442-53.

Drossman, D. A., Chang, L., Bellamy, N., Gallo-Torres, H. E., Lembo, A., Mearin, F., Norton, N. J. & Whorwell, P. 2011. Severity in irritable bowel syndrome: a Rome Foundation Working Team report. *Am J Gastroenterol,* 106, 1749-59.

Drossman, D. A., Morris, C. B., Schneck, S., Hu, Y. J., Norton, N. J., Norton, W. F., Weinland, S. R., Dalton, C., Leserman, J. & Bangdiwala, S. I. 2009. International survey of patients with IBS: symptom features and their severity, health status, treatments, and risk taking to achieve clinical benefit. *J Clin Gastroenterol,* 43, 541-50.

Drossman, D. A., Patrick, D. L., Whitehead, W. E., Toner, B. B., Diamant, N. E., Hu, Y., Jia, H. & Bangdiwala, S. I. 2000. Further validation of the IBS-QOL: a disease-specific quality-of-life questionnaire. *Am J Gastroenterol,* 95, 999-1007.

Duboc, H., Rainteau, D., Rajca, S., Humbert, L., Farabos, D., Maubert, M., Grondin, V., Jouet, P., Bouhassira, D., Seksik, P., Sokol, H., Coffin, B. & Sabate, J. M. 2012. Increase in fecal primary bile acids and dysbiosis in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol Motil,* 24, 513-20, e246-7.

Duncan, S. H., Belenguer, A., Holtrop, G., Johnstone, A. M., Flint, H. J. & Lobley, G. E. 2007. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol,* 73, 1073-8.

Duncan, S. H., Holtrop, G., Lobley, G. E., Calder, A. G., Stewart, C. S. & Flint, H. J. 2004. Contribution of acetate to butyrate formation by human faecal bacteria. *Br J Nutr,* 91, 915-23.

Dunn, S., Datta, A., Kallis, S., Law, E., Myers, C. E. & Whelan, K. 2011. Validation of a food frequency questionnaire to measure intakes of inulin and oligofructose. *Eur J Clin Nutr,* 65, 402-8.

DuPont, A. W., Jiang, Z. D., Harold, S. A., Snyder, N., Galler, G. W., Garcia-Torres, F. & DuPont, H. L. 2014. Motility abnormalities in irritable bowel syndrome. *Digestion,* 89, 119-23.

Eickhoff, J. C. 2008. Placebo effect-adjusted assessment of quality of life in placebo-controlled clinical trials. *Stat Med,* 27, 1387-402.

Elia, M. & Cummings, J. H. 2007. Physiological aspects of energy metabolism and gastrointestinal effects of carbohydrates. *Eur J Clin Nutr,* 61 Suppl 1, S40-74.

Elsenbruch, S. & Enck, P. 2015. Placebo effects and their determinants in gastrointestinal disorders. *Nat Rev Gastroenterol Hepatol,* 12, 472-85.

Enck, P., Junne, F., Klosterhalfen, S., Zipfel, S. & Martens, U. 2010. Therapy options in irritable bowel syndrome. *Eur J Gastroenterol Hepatol,* 22, 1402-11.

Engsbro, A. L., Simren, M. & Bytzer, P. 2012. Short-term stability of subtypes in the irritable bowel syndrome: prospective evaluation using the Rome III classification. *Aliment Pharmacol Ther,* 35, 350-9.

Eswaran, S., Muir, J. & Chey, W. D. 2013. Fiber and functional gastrointestinal disorders. *Am J Gastroenterol,* 108, 718-27.

Fallingborg, J., Christensen, L. A., Ingeman-Nielsen, M., Jacobsen, B. A., Abildgaard, K. & Rasmussen, H. H. 1989. pH-profile and regional transit times of the normal gut measured by a radiotelemetry device. *Aliment Pharmacol Ther,* 3, 605-13.

Farmer, A. D., Scott, S. M. & Hobson, A. R. 2013. Gastrointestinal motility revisited: The wireless motility capsule. *United European Gastroenterol J,* 1, 413-21.

Fava, F., Gitau, R., Griffin, B. A., Gibson, G. R., Tuohy, K. M. & Lovegrove, J. A. 2013. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population. *Int J Obes,* 37(2), 216-23.

Food and Drug Administration (FDA) 2012. Guidance for industry: Irritable bowel syndrome - Clinical evaluation of drugs for treatment. US department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, USA. Retrieved from: http://www.fda.gov/downloads/Drugs/.../Guidances/UCM205269.pdf

Fernandez-Banares, F., Rosinach, M., Esteve, M., Forne, M., Espinos, J. C. & Maria Viver, J. 2006. Sugar malabsorption in functional abdominal bloating: a pilot study on the long-term effect of dietary treatment. *Clin Nutr,* 25, 824-31.

Flint, H. J., Scott, K. P., Louis, P. & Duncan, S. H. 2012. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol,* 9, 577-89.

Fontana, L., Bermudez-Brito, M., Plaza-Diaz, J., Muñoz-Quezada, S. & Gil, A. 2013. Sources, isolation, characterisation and evaluation of probiotics. *Br J Nutr,* 109 Suppl 2, S35-50.

Food Standards Agency 1994. *Food portion sizes,* London, UK, The Stationery Office.

Food Standards Agency 2002. *McCance and Widdowson's The Composition of Foods,* Cambridge, UK, The Royal Society of Chemistry.

Ford, A. C., Moayyedi, P., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., Soffer, E. E., Spiegel, B. M. & Quigley, E. M. 2014a. American College of Gastroenterology monograph on the management of irritable bowel syndrome and chronic idiopathic constipation. *Am J Gastroenterol,* 109 Suppl 1, S2-26.

Ford, A. C., Quigley, E. M., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., Soffer, E. E., Spiegel, B. M. & Moayyedi, P. 2014b. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Am J Gastroenterol,* 109, 1547-61.

Ford, A. C., Talley, N. J., Spiegel, B. M., Foxx-Orenstein, A. E., Schiller, L., Quigley, E. M. & Moayyedi, P. 2008. Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: systematic review and meta-analysis. *BMJ,* 337, a2313.

Fordtran, J. S., Rector, F. C., Locklear, T. W. & Ewton, M. F. 1967. Water and solute movement in the small intestine of patients with sprue. *J Clin Invest,* 46, 287-98.

Fraher, M. H., O'Toole, P. W. & Quigley, E. M. 2012. Techniques used to characterize the gut microbiota: a guide for the clinician. *Nat Rev Gastroenterol Hepatol,* 9, 312-22.

Francis, C. Y., Morris, J. & Whorwell, P. J. 1997. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther,* 11, 395-402.

Freudenheim, J. L. 1999. Study design and hypothesis testing: issues in the evaluation of evidence from research in nutritional epidemiology. *Am J Clin Nutr,* 69, 1315s-21s.

Fritscher-Ravens, A., Schuppan, D., Ellrichmann, M., Schoch, S., Rocken, C., Brasch, J., Bethge, J., Bottner, M., Klose, J. & Milla, P. J. 2014. Confocal endomicroscopy shows food-associated changes in the intestinal mucosa of patients with irritable bowel syndrome. *Gastroenterology,* 147, 1012-20.e4.

Fujimoto, J., Matsuki, T., Sasamoto, M., Tomii, Y. & Watanabe, K. 2008. Identification and quantification of Lactobacillus casei strain Shirota in human feces with strain-specific primers derived from randomly amplified polymorphic DNA. *Int J Food Microbiol,* 126, 210-5.

Gao, J., Gillilland, M. G. & Owyang, C. 2014. Rifaximin, gut microbes and mucosal inflammation: unraveling a complex relationship. *Gut Microbes,* 5, 571-5.

Garratt, A., Schmidt, L., Mackintosh, A. & Fitzpatrick, R. 2002. Quality of life measurement: bibliographic study of patient assessed health outcome measures. *BMJ,* 324, 1417.

Gearry, R. B., Irving, P. M., Barrett J.S., Nathan, D.M., Shepherd, S.J. & Gibson, P.R. 2009. Reduction of dietary poorly absorbed short-chain carbohydrates (FODMAPs) improves abdominal symptoms in patients with inflammatory bowel disease - a pilot study. *J Crohn's Colitis*, 3, 8-14.

Gibson, G. R., Scott, K.P., Rastall, R.A., Tuohy, K.M., Hotchkiss, A., Dubert-Ferrandon, A., Gareau, M., Murphy, E. F., Saulnier, D., Loh, G., Macfarlane, S., Delzenne, N., Ringel, Y., Kozianowski, G., Dickmann, R., Lenoir-Wijnkook, I., Walker, C. & Buddington, R. 2010. Dietary prebiotics: current status and new definition. *Food Sci Technol Bull: Functional Foods,* 7, 1-19.

Glanz, K. 1980. Compliance with dietary regimens: its magnitude, measurement, and determinants. *Prev Med,* 9, 787-804.

Goldstein, R., Braverman, D. & Stankiewicz, H. 2000. Carbohydrate malabsorption and the effect of dietary restriction on symptoms of irritable bowel syndrome and functional bowel complaints. *Isr Med Assoc J,* 2, 583-587.

Gorzelak, M. A., Gill, S. K., Tasnim, N., Ahmadi-Vand, Z., Jay, M. & Gibson, D. L. 2015. Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool. *PLoS One,* 10, e0134802.

Gougoulias, C., Sandaradura, S., Meng, X., Perz, A. C., Leeds, A. R. & Thomas, L. V. 2008. Changes in the intestinal microbiota after a short period of dietary over-indulgence, representative of a holiday or festival season. *Food Sci Technol Bull: Functional Foods,* 5, 51-59.

Graf, D., Di Cagno, R., Fak, F., Flint, H. J., Nyman, M., Saarela, M. & Watzl, B. 2015. Contribution of diet to the composition of the human gut microbiota. *Microb Ecol Health Dis,* 26, 26164.

Gralnek, I. M., Hays, R. D., Kilbourne, A., Naliboff, B. & Mayer, E. A. 2000. The impact of irritable bowel syndrome on health-related quality of life. *Gastroenterology,* 119, 654-60.

Gritz, E.C. & Bhandari, V. 2015. The human neonatal gut microbiome: a brief review. *Front Pediatr*, 3,17.

Gunnarsson, J. & Simren, M. 2008. Efficient diagnosis of suspected functional bowel disorders. *Nat Clin Pract Gastroenterol Hepatol,* 5, 498-507.

Gunnarsson, J. & Simren, M. 2009. Peripheral factors in the pathophysiology of irritable bowel syndrome. *Dig Liver Dis,* 41, 788-93.

Gupta, S. K. 2011. Intention-to-treat concept: A review. *Perspect Clin Res,* 2, 109-12.

Guyatt, G. H., Feeny, D. H. & Patrick, D. L. 1993. Measuring health-related quality of life. *Ann Intern Med,* 118, 622-9.

Halmos, E. P., Christophersen, C. T., Bird, A. R., Shepherd, S. J., Gibson, P. R. & Muir, J. G. 2015. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. *Gut,* 64, 93-100.

Halmos, E. P., Power, V. A., Shepherd, S. J., Gibson, P. R. & Muir, J. G. 2014. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology,* 146, 67-75.e5.

Halpert, A., Dalton, C. B., Palsson, O., Morris, C., Hu, Y., Bangdiwala, S., Hankins, J., Norton, N. & Drossman, D. 2007. What patients know about irritable bowel syndrome (IBS) and what they would like to know. National Survey on Patient Educational Needs in IBS and development and validation of the Patient Educational Needs Questionnaire (PEQ). *Am J Gastroenterol,* 102, 1972-1982.

Harvie, R., Chisholm, A. & M, S. 2013. A reduction in FODMAP intake correlates strongly with a reduction in IBS symptoms: the FIBS study. *J Gastroenterol Hepatol,* 28, 352.

Hayes, P., Corish, C., O'Mahony, E. & Quigley, E. M. 2014. A dietary survey of patients with irritable bowel syndrome. *J Hum Nutr Diet,* 27 Suppl 2, 36-47.

Heaton, K. W., Radvan, J., Cripps, H., Mountford, R. A., Braddon, F. E. & Hughes, A. O. 1992. Defecation frequency and timing, and stool form in the general population: a prospective study. *Gut,* 33, 818-24.

Heitkemper, M. M., Jarrett, M., Cain, K. C., Shaver, J., Walker, E. & Lewis, L. 1995. Daily gastrointestinal symptoms in women with and without a diagnosis of IBS. *Dig Dis Sci,* 40, 1511-9.

Hernando-Harder, A. C., Serra, J., Azpiroz, F., Mila, M., Aguade, S., Malagelada, C., Tremolaterra, F., Villoria, A. & Malagelada, J. R. 2010. Colonic responses to gas loads in subgroups of patients with abdominal bloating. *Am J Gastroenterol,* 105, 876-82.

Hernot, D. C., Boileau, T. W., Bauer, L. L., Middelbos, I. S., Murphy, M. R., Swanson, K. S. & Fahey, G. C., Jr. 2009. In vitro fermentation profiles, gas production rates, and microbiota modulation as affected by certain fructans, galactooligosaccharides, and polydextrose. *J Agric Food Chem,* 57, 1354-61.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C. & Sanders, M. E. 2014. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol,* 11, 506-14.

Holscher, H. D., Caporaso, J. G., Hooda, S., Brulc, J. M., Fahey, G. C., Jr. & Swanson, K. S. 2015. Fiber supplementation influences phylogenetic structure and functional capacity of the human intestinal microbiome: follow-up of a randomized controlled trial. *Am J Clin Nutr,* 101, 55-64.

Hou, X., Chen, S., Zhang, Y., Sha, W., Yu, X., Elsawah, H., Afifi, A. F., El-Khayat, H. R., Nouh, A., Hassan, M. F., Fatah, A. A., Rucker Joerg, I., Sanchez Nunez, J. M., Osthoff Rueda, R., Jurkowska, G., Walczak, M*., et al.* 2014. Quality of life in patients with Irritable Bowel Syndrome (IBS), assessed using the IBS-Quality of Life (IBS-QOL) measure after 4 and 8 weeks of treatment with mebeverine hydrochloride or pinaverium bromide: results of an international prospective observational cohort study in Poland, Egypt, Mexico and China. *Clin Drug Investig,* 34, 783-93.

Hung, A., Kang, N., Bollom, A., Wolf, J. L. & Lembo, A. 2015. Complementary and Alternative Medicine Use Is Prevalent Among Patients with Gastrointestinal Diseases. *Dig Dis Sci*, 60(7), 1883-8.

Hungin, A. P., Becher, A., Cayley, B., Heidelbaugh, J. J., Muris, J. W., Rubin, G., Seifert, B., Russell, A. & De Wit, N. J. 2015. Irritable bowel syndrome: an integrated explanatory model for clinical practice. *Neurogastroenterol Motil,* 27, 750-63.

Hungin, A. P., Molloy-Bland, M., Claes, R., Heidelbaugh, J., Cayley, W. E., Jr., Muris, J., Seifert, B., Rubin, G. & de Wit, N. 2014. Systematic review: the perceptions, diagnosis and management of irritable bowel syndrome in primary care--a Rome Foundation working team report. *Aliment Pharmacol Ther,* 40, 1133-45.

Hungin, A. P., Mulligan, C., Pot, B., Whorwell, P., Agreus, L., Fracasso, P., Lionis, C., Mendive, J., Philippart de Foy, J. M., Rubin, G., Winchester, C. & de Wit, N. 2013. Systematic review: probiotics in the management of lower gastrointestinal symptoms in clinical practice -an evidence-based international guide. *Aliment Pharmacol Ther,* 38, 864-86.

Hungin, A. P., Whorwell, P. J., Tack, J. & Mearin, F. 2003. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. *Aliment Pharmacol Ther,* 17, 643-50.

Hunter, J. O., Tuffnell, Q. & Lee, A. J. 1999. Controlled trial of oligofructose in the management of irritable bowel syndrome. *J Nutr,* 129, 1451S-3S.

Hyams, J. S. 1983. Sorbitol intolerance: an unappreciated cause of functional gastrointestinal complaints. *Gastroenterology,* 84, 30-33.

ICH 1998. Statistical principles for Clinical Trials E9.) ICH Harmonised Tripartite Guideline.

Illner, A. K., Freisling, H., Boeing, H., Huybrechts, I., Crispim, S. P. & Slimani, N. 2012. Review and evaluation of innovative technologies for measuring diet in nutritional epidemiology. *Int J Epidemiol,* 41, 1187-203.

Irvine, E. J., Whitehead, W. E., Chey, W. D., Matsueda, K., Shaw, M., Talley, N. J. & Veldhuyzen van Zanten, S. J. 2006. Design of treatment trials for functional gastrointestinal disorders. *Gastroenterology,* 130, 1538-51.

Jackson, J. A., Kinn, S. & Dalgarno, P. 2005. Patient-centred outcomes in dietary research. *J Hum Nutr Diet,* 18, 83-92.

Jalanka, J., Salonen, A., Salojarvi, J., Ritari, J., Immonen, O., Marciani, L., Gowland, P., Hoad, C., Garsed, K., Lam, C., Palva, A., Spiller, R. C. & de Vos, W. M. 2015. Effects of bowel cleansing on the intestinal microbiota. *Gut,* 64, 1562-8.

Jalanka-Tuovinen, J., Salojarvi, J., Salonen, A., Immonen, O., Garsed, K., Kelly, F. M., Zaitoun, A., Palva, A., Spiller, R. C. & de Vos, W. M. 2014. Faecal microbiota composition and host-microbe cross-talk following gastroenteritis and in postinfectious irritable bowel syndrome. *Gut,* 63, 1737-45.

Jalanka-Tuovinen, J., Salonen, A., Nikkila, J., Immonen, O., Kekkonen, R., Lahti, L., Palva, A. & de Vos, W. M. 2011. Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. *PLoS One,* 6, e23035.

Jeffery, I. B., O'Toole, P. W., Ohman, L., Claesson, M. J., Deane, J., Quigley, E. M. & Simren, M. 2012. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut,* 61, 997-1006.

Jones, H. F., Butler, R. N. & Brooks, D. A. 2011. Intestinal fructose transport and malabsorption in humans. *Am J Physiol Gastrointest Liver Physiol,* 300, G202-6.

Jones, J., Boorman, J., Cann, P., Forbes, A., Gomborone, J., Heaton, K., Hungin, P., Kumar, D., Libby, G., Spiller, R., Read, N., Silk, D. & Whorwell, P. 2000. British Society of Gastroenterology guidelines for the management of the irritable bowel syndrome. *Gut,* 47 Suppl 2, ii1-19.

Kannampalli, P., Shaker, R. & Sengupta, J. N. 2011. Colonic butyrate- algesic or analgesic? *Neurogastroenterol Motil,* 23, 975-9.

Kashyap, P. C., Marcobal, A., Ursell, L. K., Larauche, M., Duboc, H., Earle, K. A., Sonnenburg, E. D., Ferreyra, J. A., Higginbottom, S. K., Million, M., Tache, Y., Pasricha, P. J., Knight, R., Farrugia, G. & Sonnenburg, J. L. 2013. Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. *Gastroenterology,* 144, 967-77.

Kassinen, A., Krogius-Kurikka, L., Makivuokko, H., Rinttila, T., Paulin, L., Corander, J., Malinen, E., Apajalahti, J. & Palva, A. 2007. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology,* 133, 24-33.

Kerckhoffs, A. P., Samsom, M., van der Rest, M. E., de Vogel, J., Knol, J., Ben-Amor, K. & Akkermans, L. M. 2009. Lower Bifidobacteria counts in both duodenal mucosa-associated and fecal microbiota in irritable bowel syndrome patients. *World J Gastroenterol,* 15, 2887-92.

Kim, H. J., Camilleri, M., McKinzie, S., Lempke, M. B., Burton, D. D., Thomforde, G. M. & Zinsmeister, A. R. 2003. A randomized controlled trial of a probiotic, VSL#3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther,* 17, 895-904.

Kim, H. J., Roque, M. I. V., Camilleri, M., Stephens, D., Burton, D. D., Baxter, K., Thomforde, G. & Zinsmeister, A. R. 2005. A randomized controlled trial of a probiotic combination VSL# 3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterol Motil,* 17, 687-696.

Kim, J., Kwon, J., Noh, G. & Lee, S. S. 2013. The effects of elimination diet on nutritional status in subjects with atopic dermatitis. *Nutr Res Pract,* 7, 488-94.

Kim, S. E., Choi, S. C., Park, K. S., Park, M. I., Shin, J. E., Lee, T. H., Jung, K. W., Koo, H. S. & Myung, S. J. 2015. Change of Fecal Flora and Effectiveness of the Short-term VSL#3 Probiotic Treatment in Patients With Functional Constipation. *J Neurogastroenterol Motil,* 21, 111-20.

King, T. S., Elia, M. & Hunter, J. O. 1998. Abnormal colonic fermentation in irritable bowel syndrome. *Lancet,* 352, 1187-9.

Knights, D., Ward, T. L., McKinlay, C. E., Miller, H., Gonzalez, A., McDonald, D. & Knight, R. 2014. Rethinking "enterotypes". *Cell Host Microbe,* 16, 433-7.

Koloski, N. A., Boyce, P. M., Jones, M. P. & Talley, N. J. 2012. What level of IBS symptoms drives impairment in health-related quality of life in community subjects with irritable bowel syndrome? Are current IBS symptom thresholds clinically meaningful? *Qual Life Res,* 21, 829-36.

Kramer, M. S. & Shapiro, S. H. 1984. Scientific challenges in the application of randomized trials. *JAMA,* 252, 2739-45.

Krogius-Kurikka, L., Lyra, A., Malinen, E., Aarnikunnas, J., Tuimala, J., Paulin, L., Makivuokko, H., Kajander, K. & Palva, A. 2009. Microbial community analysis reveals high level phylogenetic alterations in the overall gastrointestinal microbiota of diarrhoea-predominant irritable bowel syndrome sufferers. *BMC Gastroenterol,* 9, 95.

Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonak, J., Lind, K., Sindelka, R., Sjoback, R., Sjogreen, B., Strombom, L., Stahlberg, A. & Zoric, N. 2006. The real-time polymerase chain reaction. *Mol Aspects Med,* 27, 95-125.

Labus, J., Gupta, A., Gill, H. K., Posserud, I., Mayer, M., Raeen, H., Bolus, R., Simren, M., Naliboff, B. D. & Mayer, E. A. 2013. Randomised clinical trial: symptoms of the irritable bowel syndrome are improved by a psycho-education group intervention. *Aliment Pharmacol Ther,* 37, 304-15.

Lackner, J. M., Jaccard, J., Keefer, L., Firth, R., Carosella, A. M., Sitrin, M. & Brenner, D. 2014. The accuracy of patient-reported measures for GI symptoms: a comparison of real time and retrospective reports. *Neurogastroenterol Motil,* 26, 1802-11.

Lacy, B. E., Lembo, A. J., Macdougall, J. E., Shiff, S. J., Kurtz, C. B., Currie, M. G. & Johnston, J. M. 2014. Responders vs clinical response: a critical analysis of data from linaclotide phase 3 clinical trials in IBS-C. *Neurogastroenterol Motil,* 26, 326-33.

Ladabaum, U., Boyd, E., Zhao, W. K., Mannalithara, A., Sharabidze, A., Singh, G., Chung, E. & Levin, T. R. 2012. Diagnosis, comorbidities, and management of irritable bowel syndrome in patients in a large health maintenance organization. *Clin Gastroenterol Hepatol,* 10, 37-45.

Lauber, C. L., Zhou, N., Gordon, J. I., Knight, R. & Fierer, N. 2010. Effect of storage conditions on the assessment of bacterial community structure in soil and human-associated samples. *FEMS Microbiol Lett,* 307, 80-6.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J. M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jorgensen, T., Brandslund, I*., et al.* 2013. Richness of human gut microbiome correlates with metabolic markers. *Nature,* 500, 541-6.

Lea, R. & Whorwell, P. J. 2001. Quality of life in irritable bowel syndrome. *Pharmacoeconomics,* 19, 643-53.

Ledochowski, M., Widner, B., Bair, H., Probst, T. & Fuchs, D. 2000. Fructose- and sorbitol-reduced diet improves mood and gastrointestinal disturbances in fructose malabsorbers. *Scand J Gastroenterol,* 35, 1048-1052.

Lee, J. H. & O'Sullivan, D. J. 2010. Genomic insights into bifidobacteria. *Microbiol Mol Biol Rev,* 74, 378-416.

Lepage, P., Seksik, P., Sutren, M., de la Cochetiere, M. F., Jian, R., Marteau, P. & Dore, J. 2005. Biodiversity of the mucosa-associated microbiota is stable along the distal digestive tract in healthy individuals and patients with IBD. *Inflamm Bowel Dis,* 11, 473-80.

Ley, R. E., Turnbaugh, P. J., Klein, S. & Gordon, J. I. 2006. Microbial ecology: human gut microbes associated with obesity. *Nature,* 444, 1022-23.

Li, F., Hullar, M. A., Schwarz, Y. & Lampe, J. W. 2009. Human gut bacterial communities are altered by addition of cruciferous vegetables to a controlled fruit- and vegetable-free diet. *J Nutr,* 139, 1685-1691.

Lindsay, K. L., Brennan, L. & McAuliffe, F. M. 2014. Acceptability of and compliance with a probiotic capsule intervention in pregnancy. *Int J Gynaecol Obstet,* 125, 279-80.

Lomer, M. C., Parkes, G. C. & Sanderson, J. D. 2008. Review article: lactose intolerance in clinical practice--myths and realities. *Aliment Pharmacol Ther,* 27, 93-103.

Longstreth, G. F., Thompson, W. G., Chey, W. D., Houghton, L. A., Mearin, F. & Spiller, R. C. 2006. Functional bowel disorders. *Gastroenterology,* 130, 1480-1491.

Lovell, R. M. & Ford, A. C. 2012. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol,* 10, 712-721 e4.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. 2012. Diversity, stability and resilience of the human gut microbiota. *Nature,* 489, 220-30.

Ludidi, S., Conchillo, J. M., Keszthelyi, D., Van Avesaat, M., Kruimel, J. W., Jonkers, D. M. & Masclee, A. A. 2012. Rectal hypersensitivity as hallmark for irritable bowel syndrome: defining the optimal cutoff. *Neurogastroenterol Motil,* 24, 729-735.

Ludidi, S., Jonkers, D. M., Koning, C. J., Kruimel, J. W., Mulder, L., van der Vaart, I. B., Conchillo, J. M. & Masclee, A. A. 2014. Randomized clinical trial on the effect of a multispecies probiotic on visceroperception in hypersensitive IBS patients. *Neurogastroenterol Motil,* 26, 705-14.

Lyra, A., Rinttila, T., Nikkila, J., Krogius-Kurikka, L., Kajander, K., Malinen, E., Matto, J., Makela, L. & Palva, A. 2009. Diarrhoea-predominant irritable bowel syndrome distinguishable by 16S rRNA gene phylotype quantification. *World J Gastroenterol,* 15, 5936-45.

Ma, Y., Olendzki, B. C., Pagoto, S. L., Hurley, T. G., Magner, R. P., Ockene, I. S., Schneider, K. L., Merriam, P. A. & Hebert, J. R. 2009. Number of 24-hour diet recalls needed to estimate energy intake. *Ann Epidemiol,* 19, 553-9.

Macfarlane, S., Macfarlane, G. T. & Cummings, J. H. 2006. Review article: prebiotics in the gastrointestinal tract. *Aliment Pharmacol Ther,* 24, 701-14.

Madsen, J. L., Linnet, J. & Rumessen, J. J. 2006. Effect of nonabsorbed amounts of a fructose-sorbitol mixture on small intestinal transit in healthy volunteers. *Dig Dis Sci,* 51, 147-53.

Majid, H. A., Emery, P. W. & Whelan, K. 2012. Definitions, attitudes, and management practices in relation to diarrhea during enteral nutrition: a survey of patients, nurses, and dietitians. *Nutr Clin Pract,* 27, 252-60.

Major, G., Krishnasamy, S., Mulvenna, C., Pritchard, S., Hoad, C., Marciani, L., Lomer, M., Gowland, P. & Spiller, R. 2015a. Effect of the low FODMAP diet and oligofructose supplement on colonic volume, transit and fermentation: A double-blind randomised controlled trial using magnetic resonance imaging in healthy volunteers. *Gut,* 64 Suppl 1, A57.

Major, G., Pritchard, S., Murray, K., Hoad, C., Marciani, L., Gowland, P. & Spiller, R. 2015b. Mechanisms underlying FODMAP-induced symptoms in patients with irritable bowel syndrome: a double-blind crossover trial using magnetic resonance imaging. *Gut,* 64 Suppl 1, A33.

Malinen, E., Rinttila, T., Kajander, K., Matto, J., Kassinen, A., Krogius, L., Saarela, M., Korpela, R. & Palva, A. 2005. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol,* 100, 373-382.

Manabe, N., Wong, B. S., Camilleri, M., Burton, D., McKinzie, S. & Zinsmeister, A. R. 2010. Lower functional gastrointestinal disorders: evidence of abnormal colonic transit in a 287 patient cohort. *Neurogastroenterol Motil,* 22, 293-e82.

Manichanh, C., Eck, A., Varela, E., Roca, J., Clemente, J. C., Gonzalez, A., Knights, D., Knight, R., Estrella, S., Hernandez, C., Guyonnet, D., Accarino, A., Santos, J., Malagelada, J. R., Guarner, F. & Azpiroz, F. 2014. Anal gas evacuation and colonic microbiota in patients with flatulence: effect of diet. *Gut,* 63, 401-8.

Marciani, L., Cox, E. F., Hoad, C. L., Pritchard, S., Totman, J. J., Foley, S., Mistry, A., Evans, S., Gowland, P. A. & Spiller, R. C. 2010. Postprandial changes in small bowel water content in healthy subjects and patients with irritable bowel syndrome. *Gastroenterology,* 138, 469-77.

Marriott, B. P., Cole, N. & Lee, E. 2009. National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. *J Nutr,* 139, 1228s-1235s.

Marsh, A., Eslick, E. M. & Eslick, G. D. 2015. Does a diet low in FODMAPs reduce symptoms associated with functional gastrointestinal disorders? A comprehensive systematic review and meta-analysis. *Eur J Nutr*, Epub ahead of print.

Marshall, J. K., Thabane, M., Garg, A. X., Clark, W. F., Moayyedi, P. & Collins, S. M. 2010. Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery. *Gut,* 59, 605-11.

Marshall, J. K., Thabane, M., Garg, A. X., Clark, W. F., Salvadori, M. & Collins, S. M. 2006. Incidence and epidemiology of irritable bowel syndrome after a large waterborne outbreak of bacterial dysentery. *Gastroenterology,* 131, 445-50.

Matricon, J., Meleine, M., Gelot, A., Piche, T., Dapoigny, M., Muller, E. & Ardid, D. 2012. Review article: Associations between immune activation, intestinal permeability and the irritable bowel syndrome. *Aliment Pharmacol Ther,* 36, 1009-31.

Matsuki, T., Watanabe, K., Fujimoto, J., Miyamoto, Y., Takada, T., Matsumoto, K., Oyaizu, H. & Tanaka, R. 2002. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Appl Environ Microbiol,* 68, 5445-51.

Matto, J., Maunuksela, L., Kajander, K., Palva, A., Korpela, R., Kassinen, A. & Saarela, M. 2005. Composition and temporal stability of gastrointestinal microbiota in irritable bowel syndrome--a longitudinal study in IBS and control subjects. *FEMS Immunol Med Microbiol,* 43, 213-22.

Maukonen, J., Satokari, R., Matto, J., Soderlund, H., Mattila-Sandholm, T. & Saarela, M. 2006. Prevalence and temporal stability of selected clostridial groups in irritable bowel syndrome in relation to predominant faecal bacteria. *J Med Microbiol,* 55, 625-33.

Maxwell, P. R., Rink, E., Kumar, D. & Mendall, M. A. 2002. Antibiotics increase functional abdominal symptoms. *Am J Gastroenterol,* 97, 104-8.

Mayer, E. A., Naliboff, B. D. & Craig, A. D. 2006. Neuroimaging of the brain-gut axis: from basic understanding to treatment of functional GI disorders. *Gastroenterology,* 131, 1925-42.

Mazzawi, T., Hausken, T., Gundersen, D. & El-Salhy, M. 2013. Effects of dietary guidance on the symptoms, quality of life and habitual dietary intake of patients with irritable bowel syndrome. *Mol Med Rep,* 8, 845-52.

McAlister, F. A., Straus, S. E., Sackett, D. L. & Altman, D. G. 2003. Analysis and reporting of factorial trials: a systematic review. *JAMA,* 289, 2545-53.

McColl, E. 2004. Best practice in symptom assessment: a review. *Gut,* 53 Suppl 4, iv49-54.

McCoubrey, H., Parkes, G. C., Sanderson, J. D. & Lomer, M. C. E. 2008. Nutritional intakes in irritable bowel syndrome. *J Hum Nutr Diet,* 21, 396.

McKenzie, Y. A., Alder, A., Anderson, W., Wills, A., Goddard, L., Gulia, P., Jankovich, E., Mutch, P., Reeves, L. B., Singer, A. & Lomer, M. C. 2012. British Dietetic Association evidence-based guidelines for the dietary management of irritable bowel syndrome in adults. *J Hum Nutr Diet,* 25, 260-74.

Mdege, N. D., Brabyn, S., Hewitt, C., Richardson, R. & Torgerson, D. J. 2014. The 2 x 2 cluster randomized controlled factorial trial design is mainly used for efficiency and to explore intervention interactions: a systematic review. *J Clin Epidemiol,* 67, 1083-92.

Mearin, F., Baro, E., Roset, M., Badia, X., Zarate, N. & Perez, I. 2004. Clinical patterns over time in irritable bowel syndrome: symptom instability and severity variability. *Am J Gastroenterol,* 99, 113-21.

Menees, S. B., Maneerattannaporn, M., Kim, H. M. & Chey, W. D. 2012. The efficacy and safety of rifaximin for the irritable bowel syndrome: a systematic review and meta-analysis. *Am J Gastroenterol,* 107, 28-35.

Mercer, M., Brinich, M. A., Geller, G., Harrison, K., Highland, J., James, K., Marshall, P., McCormick, J. B., Tilburt, J., Achkar, J. P., Farrell, R. M. & Sharp, R. R. 2012. How patients view probiotics: findings from a multicenter study of patients with inflammatory bowel disease and irritable bowel syndrome. *J Clin Gastroenterol,* 46, 138-44.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J. F., Rougeot, C., Pichelin, M., Cazaubiel, M. & Cazaubiel, J. M. 2011. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *Br J Nutr,* 105, 755-64.

Michail, S. & Kenche, H. 2011. Gut microbiota is not modified by Randomized, Double-blind, Placebo-controlled Trial of VSL#3 in Diarrhea-predominant Irritable Bowel Syndrome. *Probiotics Antimicrob Proteins,* 3, 1-7.

Miller, L. E. & Ouwehand, A. C. 2013. Probiotic supplementation decreases intestinal transit time: meta-analysis of randomized controlled trials. *World J Gastroenterol,* 19, 4718-25.

Millet, S., Van Oeckel, M. J., Aluwe, M., Delezie, E. & De Brabander, D. L. 2010. Prediction of in vivo short-chain fatty acid production in hindgut fermenting mammals: problems and pitfalls. *Crit Rev Food Sci Nutr,* 50, 605-19.

Miquel, S., Martin, R., Rossi, O., Bermudez-Humaran, L. G., Chatel, J. M., Sokol, H., Thomas, M., Wells, J. M. & Langella, P. 2013. Faecalibacterium prausnitzii and human intestinal health. *Curr Opin Microbiol,* 16, 255-61.

Mitchell, N., Hewitt, C. E., Jayakody, S., Islam, M., Adamson, J., Watt, I. & Torgerson, D. J. 2011. Randomised controlled trial of food elimination diet based on IgG antibodies for the prevention of migraine like headaches. *Nutr J,* 10, 85.

Moayyedi, P., Quigley, E. M., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., Soffer, E. E., Spiegel, B. M. & Ford, A. C. 2014. The effect of fiber supplementation on irritable bowel syndrome: a systematic review and meta-analysis. *Am J Gastroenterol,* 109, 1367-74.

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gotzsche, P. C., Devereaux, P. J., Elbourne, D., Egger, M. & Altman, D. G. 2010. CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials. *J Clin Epidemiol,* 63, e1-37.

Montalto, M., D'Onofrio, F., Gallo, A., Cazzato, A. & Gasbarrini, G. 2009. Intestinal microbiota and its functions. *Dig Liv Dis*, 3, 30–34.

Muir, J. G., Rose, R., Rosella, O., Liels, K., Barrett, J. S., Shepherd, S. J. & Gibson, P. R. 2009. Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *J Agric Food Chem,* 57, 554-65.

Muir, J. G., Shepherd, S. J., Rosella, O., Rose, R., Barrett, J. S. & Gibson, P. R. 2007. Fructan and free fructose content of common Australian vegetables and fruit. *J Agric Food Chem,* 55, 6619-27.

Mujagic, Z., Keszthelyi, D., Aziz, Q., Reinisch, W., Quetglas, E. G., De Leonardis, F., Segerdahl, M. & Masclee, A. A. 2015. Systematic review: instruments to assess abdominal pain in irritable bowel syndrome. *Aliment Pharmacol Ther,* 42, 1064-81.

Murray, K., Wilkinson-Smith, V., Hoad, C., Costigan, C., Cox, E., Lam, C., Marciani, L., Gowland, P. & Spiller, R. C. 2014. Differential effects of FODMAPs (fermentable oligo-, di-, mono-saccharides and polyols) on small and large intestinal contents in healthy subjects shown by MRI. *Am J Gastroenterol,* 109, 110-9.

Naliboff, B. D., Fullerton, S. & Mayer, E. A. 1999. Measurement of symptoms in irritable bowel syndrome clinical trials. *Am J Med,* 107, 81s-84s.

Nanda, R., James, R., Smith, H., Dudley, C.R. & Jewell, D.P. 1989. Food intolerance and the irritable bowel syndrome. *Gut*, 30, 1099-104.

Ng, S. C., Lam, E. F., Lam, T. T., Chan, Y., Law, W., Tse, P. C., Kamm, M. A., Sung, J. J., Chan, F. K. & Wu, J. C. 2013. Effect of probiotic bacteria on the intestinal microbiota in irritable bowel syndrome. *J Gastroenterol Hepatol,* 28, 1624-31.

National Institute for Health and Care Excellence (NICE 2015). Irritable bowel syndrome in adults: diagnosis and management of irritable bowel syndrome in primary care. Retrieved from: https://www.nice.org.uk/guidance/cg61

O'Donnell, L. J., Virjee, J. & Heaton, K. W. 1990. Detection of pseudodiarrhoea by simple clinical assessment of intestinal transit rate. *BMJ,* 300, 439-40.

O'Keefe, S. J., Li, J. V., Lahti, L., Ou, J., Carbonero, F., Mohammed, K., Posma, J. M., Kinross, J., Wahl, E., Ruder, E., Vipperla, K., Naidoo, V., Mtshali, L., Tims, S., Puylaert, P. G., DeLany, J.*, et al.* 2015. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun,* 6, 6342.

O'Mahony, L., McCarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., O'Sullivan, G. C., Kiely, B., Collins, J. K., Shanahan, F. & Quigley, E. M. 2005. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology,* 128, 541-51.

Ohman, L. & Simren, M. 2010. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nat Rev Gastroenterol Hepatol,* 7, 163-73.

Ohman, L., Tornblom, H. & Simren, M. 2015. Crosstalk at the mucosal border: importance of the gut microenvironment in IBS. *Nat Rev Gastroenterol Hepatol,* 12, 36-49.

Olesen, M. & Gudmand-Hoyer, E. 2000. Efficacy, safety, and tolerability of fructooligosaccharides in the treatment of irritable bowel syndrome. *Am J Clin Nutr,* 72, 1570-5.

Ong, D. K., Mitchell, S. B., Barrett, J. S., Shepherd, S. J., Irving, P. M., Biesiekierski, J. R., Smith, S., Gibson, P. R. & Muir, J. G. 2010. Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of symptoms in irritable bowel syndrome. *J Gastroenterol Hepatol,* 25, 1366-73.

Ostgaard, H., Hausken, T., Gundersen, D. & El-Salhy, M. 2012. Diet and effects of diet management on quality of life and symptoms in patients with irritable bowel syndrome. *Mol Med Report,* 5, 1382-90.

Paineau, D., Payen, F., Panserieu, S., Coulombier, G., Sobaszek, A., Lartigau, I., Brabet, M., Galmiche, J. P., Tripodi, D., Sacher-Huvelin, S., Chapalain, V., Zourabichvili, O., Respondek, F., Wagner, A. & Bornet, F. R. 2008. The effects of regular consumption of short-chain fructo-oligosaccharides on digestive comfort of subjects with minor functional bowel disorders. *Br J Nutr,* 99, 311-8.

Parkar, S. G., Trower, T. M. & Stevenson, D. E. 2013. Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. *Anaerobe,* 23, 12-9.

Parker, T.J., Naylor, S.J., Riordan, A.M. & Hunter, J.O. 2008. Management of patients with food intolerance in irritable bowel syndrome: the development and use of an exclusion diet. *J Hum Nutr Diet*, 8, 159-66.

Parker, T. J., Woolner, J. T., Prevost, A. T., Tuffnell, Q., Shorthouse, M. & Hunter, J. O. 2001. Irritable bowel syndrome: is the search for lactose intolerance justified? *Eur J Gastroenterol Hepatol,* 13, 219-25.

Parkes, G. C., Rayment, N. B., Hudspith, B. N., Petrovska, L., Lomer, M. C., Brostoff, J., Whelan, K. & Sanderson, J. D. 2012. Distinct microbial populations exist in the mucosa-associated microbiota of sub-groups of irritable bowel syndrome. *Neurogastroenterol Motil,* 24, 31-39.

Passos, M. C., Lembo, A. J., Conboy, L. A., Kaptchuk, T. J., Kelly, J. M., Quilty, M. T., Kerr, C. E., Jacobson, E. E., Hu, R., Friedlander, E. & Drossman, D. A. 2009. Adequate relief in a treatment trial with IBS patients: a prospective assessment. *Am J Gastroenterol,* 104, 912-9.

Patrick, D. L., Drossman, D. A., Frederick, I. O., DiCesare, J. & Puder, K. L. 1998. Quality of life in persons with irritable bowel syndrome: development and validation of a new measure. *Dig Dis Sci,* 43, 400-11.

Pedersen, N., Andersen, N. N., Vegh, Z., Jensen, L., Ankersen, D. V., Felding, M., Simonsen, M. H., Burisch, J. & Munkholm, P. 2014. Ehealth: low FODMAP diet vs Lactobacillus rhamnosus GG in irritable bowel syndrome. *World J Gastroenterol,* 20, 16215-26.

Perez-Cornago, A., Martinez-Gonzalez, M. A., Ruiz-Canela, M., Jaurrieta, I., Carlos, S., Sayon-Orea, C. & Bes-Rastrollo, M. 2015. Prebiotic consumption and the incidence of overweight in a Mediterranean cohort: the Seguimiento Universidad de Navarra Project. *Am J Clin Nutr,* 102, 1554-62.

Peterlik, M. & Cross, H. S. 2009. Vitamin D and calcium insufficiency-related chronic diseases: molecular and cellular pathophysiology. *Eur J Clin Nutr,* 63, 1377-86.

Piacentino , D., Rossi, S., Alvino, V., Di Nunno, R., Piretta, L., Badiali, D., Pallotta, N. & Corazziari, E. 2015. Low FODMAP diet in irritable bowel syndrome patients offers more benefit than a low FODMAP gluten free diet in the medium and long term. Results from a double-blind randomized controlled clinical study and follow up. *Gastroenterology*, 148(4), S119.

Piche, T., Saint-Paul, M. C., Dainese, R., Marine-Barjoan, E., Iannelli, A., Montoya, M. L., Peyron, J. F., Czerucka, D., Cherikh, F., Filippi, J., Tran, A. & Hebuterne, X. 2008. Mast cells and cellularity of the colonic mucosa correlated with fatigue and depression in irritable bowel syndrome. *Gut,* 57, 468-73.

Pitz, M., Cheang, M. & Bernstein, C. N. 2005. Defining the predictors of the placebo response in irritable bowel syndrome. *Clin Gastroenterol Hepatol,* 3, 237-47.

Ponnusamy, K., Choi, J. N., Kim, J., Lee, S. Y. & Lee, C. H. 2011. Microbial community and metabolomic comparison of irritable bowel syndrome faeces. *J Med Microbiol,* 60, 817-27.

Public Health England and Food Standards Agency. 2014. National Diet and Nutrition Survey: appendices and tables. Retrieved from: https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., Cao, J.*, et al.* 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature,* 464, 59-65.

Queipo-Ortuno, M. I., Boto-Ordonez, M., Murri, M., Gomez-Zumaquero, J. M., Clemente-Postigo, M., Estruch, R., Cardona Diaz, F., Andres-Lacueva, C. & Tinahones, F. J. 2012. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *Am J Clin Nutr,* 95, 1323-34.

Rajilic-Stojanovic, M., Biagi, E., Heilig, H. G., Kajander, K., Kekkonen, R. A., Tims, S. & de Vos, W. M. 2011. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology,* 141, 1792-801.

Rajilic-Stojanovic, M., Jonkers, D. M., Salonen, A., Hanevik, K., Raes, J., Jalanka, J., de Vos, W. M., Manichanh, C., Golic, N., Enck, P., Philippou, E., Iraqi, F. A., Clarke, G., Spiller, R. C. & Penders, J. 2015. Intestinal microbiota and diet in IBS: causes, consequences, or epiphenomena? *Am J Gastroenterol,* 110, 278-87.

Ramirez-Farias, C., Slezak, K., Fuller, Z., Duncan, A., Holtrop, G. & Louis, P. 2009. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. *Br J Nutr,* 101, 541-550.

Rangel, I., Sundin, J., Fuentes, S., Repsilber, D., de Vos, W. M. & Brummer, R. J. 2015. The relationship between faecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients and healthy subjects. *Aliment Pharmacol Ther,* 42, 1211-21.

Rankin, G. & Stokes, M. 1998. Reliability of assessment tools in rehabilitation: an illustration of appropriate statistical analyses. *Clin Rehabil,* 12, 187-99.

Rao, S. S., Yu, S. & Fedewa, A. 2015. Systematic review: dietary fibre and FODMAP-restricted diet in the management of constipation and irritable bowel syndrome. *Aliment Pharmacol Ther,* 41, 1256-70.

Relman, D. A. 2012. The human microbiome: ecosystem resilience and health. *Nutr Rev,* 70 Suppl 1, S2-9.

Revicki, D. A., Wood, M., Wiklund, I. & Crawley, J. 1998. Reliability and validity of the Gastrointestinal Symptom Rating Scale in patients with gastroesophageal reflux disease. *Qual Life Res,* 7, 75-83.

Rijkers, G. T., Bengmark, S., Enck, P., Haller, D., Herz, U., Kalliomaki, M., Kudo, S., Lenoir-Wijnkoop, I., Mercenier, A., Myllyluoma, E., Rabot, S., Rafter, J., Szajewska, H., Watzl, B., Wells, J., Wolvers, D*., et al.* 2010. Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. *J Nutr,* 140, 671S-6S.

Ringel-Kulka, T., Cheng, J., Ringel, Y., Salojarvi, J., Carroll, I., Palva, A., de Vos, W. M. & Satokari, R. 2013. Intestinal microbiota in healthy U.S. young children and adults--a high throughput microarray analysis. *PLoS One,* 8, e64315.

Ringel-Kulka, T., Choi, C. H., Temas, D., Kim, A., Maier, D. M., Scott, K., Galanko, J. A. & Ringel, Y. 2015. Altered Colonic Bacterial Fermentation as a Potential Pathophysiological Factor in Irritable Bowel Syndrome. *Am J Gastroenterol,* 110, 1339-46.

Ritchie, J. 1973. Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. *Gut,* 14, 125-32.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H., Stahl, B., Guarner, F., Respondek, F., Whelan, K., Coxam, V., Davicco, M. J., Leotoing, L*., et al.* 2010. Prebiotic effects: metabolic and health benefits. *Br J Nutr,* 104 Suppl 2, S1-63.

Roberfroid, M. B. 2007. Inulin-type fructans: functional food ingredients. *J Nutr,* 137, 2493S-2502S.

Rochet, V., Rigottier-Gois, L., Ledaire, A., Andrieux, C., Sutren, M., Rabot, S., Mogenet, A., Bresson, J. L., Cools, S., Picard, C., Goupil-Feuillerat, N. & Doré, J. 2008. Survival of Bifidobacterium animalis DN-173 010 in the faecal microbiota after administration in lyophilised form or in fermented product - a randomised study in healthy adults. *J Mol Microbiol Biotechnol,* 14, 128-36.

Rochet, V., Rigottier-Gois, L., Sutren, M., Krementscki, M. N., Andrieux, C., Furet, J. P., Tailliez, P., Levenez, F., Mogenet, A., Bresson, J. L., Meance, S., Cayuela, C., Leplingard, A. & Dore, J. 2006. Effects of orally administered Lactobacillus casei DN-114 001 on the composition or activities of the dominant faecal microbiota in healthy humans. *Br J Nutr,* 95, 421-9.

Rumessen, J. J. & Gudmand-Hoyer, E. 1988. Functional bowel disease: malabsorption and abdominal distress after ingestion of fructose, sorbitol, and fructose-sorbitol mixtures. *Gastroenterology,* 95, 694-700.

Russell, D. A., Ross, R. P., Fitzgerald, G. F. & Stanton, C. 2011a. Metabolic activities and probiotic potential of bifidobacteria. *Int J Food Microbiol,* 149, 88-105.

Russell, W. R., Gratz, S. W., Duncan, S. H., Holtrop, G., Ince, J., Scobbie, L., Duncan, G., Johnstone, A. M., Lobley, G. E., Wallace, R. J., Duthie, G. G. & Flint, H. J. 2011b. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr,* 93, 1062-72.

Saito, Y. A., Locke, G. R., 3rd, Weaver, A. L., Zinsmeister, A. R. & Talley, N. J. 2005. Diet and functional gastrointestinal disorders: a population-based case-control study. *Am J Gastroenterol,* 100, 2743-8.

Salonen, A., Lahti, L., Salojarvi, J., Holtrop, G., Korpela, K., Duncan, S. H., Date, P., Farquharson, F., Johnstone, A. M., Lobley, G. E., Louis, P., Flint, H. J. & de Vos, W. M. 2014. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J,* 8, 2218-30.

Schnorr, S. L., Candela, M., Rampelli, S., Centanni, M., Consolandi, C., Basaglia, G., Turroni, S., Biagi, E., Peano, C., Severgnini, M., Fiori, J., Gotti, R., De Bellis, G., Luiselli, D., Brigidi, P.,

Mabulla, A.*, et al.* 2014. Gut microbiome of the Hadza hunter-gatherers. *Nat Commun,* 5, 3654.

Schwille-Kiuntke, J., Mazurak, N. & Enck, P. 2015. Systematic review with meta-analysis: post-infectious irritable bowel syndrome after travellers' diarrhoea. *Aliment Pharmacol Ther,* 41, 1029-37.

Scott, K. P., Antoine, J. M., Midtvedt, T. & van Hemert, S. 2015. Manipulating the gut microbiota to maintain health and treat disease. *Microb Ecol Health Dis,* 26, 25877.

Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J. & Duncan, S. H. 2013. The influence of diet on the gut microbiota. *Pharmacol Res,* 69, 52-60.

Shen, J. Z., Z. X. Mao, A. P. 2014. Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. *Inflamm Bowel Dis,* 20, 21-35.

Shepherd, S. J. & Gibson, P. R. 2006. Fructose malabsorption and symptoms of irritable bowel syndrome: guidelines for effective dietary management. *J Am Diet Assoc,* 106, 1631-9.

Shepherd, S. J. & Gibson, P. R. 2013. Nutritional inadequacies of the gluten-free diet in both recently-diagnosed and long-term patients with coeliac disease. *J Hum Nutr Diet,* 26, 349-58.

Shepherd, S. J., Parker, F. C., Muir, J. G. & Gibson, P. R. 2008. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomized placebo-controlled evidence. *Clin Gastroenterol Hepatol,* 6, 765-71.

Si, J. M., Yu, Y. C., Fan, Y. J. & Chen, S. J. 2004. Intestinal microecology and quality of life in irritable bowel syndrome patients. *World J Gastroenterol,* 10, 1802-5.

Silk, D. B., Davis, A., Vulevic, J., Tzortzis, G. & Gibson, G. R. 2009. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Ther,* 29, 508-518.

Simrén, M., Barbara, G., Flint, H. J., Spiegel, B. M., Spiller, R. C., Vanner, S., Verdu, E. F., Whorwell, P. J. & Zoetendal, E. G. 2013. Intestinal microbiota in functional bowel disorders: a Rome foundation report. *Gut*, 42(1), 159-76

Simrén, M., Ohman, L., Olsson, J., Svensson, U., Ohlson, K., Posserud, I. & Strid, H. 2010. Clinical trial: the effects of a fermented milk containing three probiotic bacteria in patients with irritable bowel syndrome - a randomized, double-blind, controlled study. *Aliment Pharmacol Ther,* 31, 218-27.

Singh, P., Staller, K., Barshop, K., Dai, E., Newman, J., Yoon, S., Castel, S. & Kuo, B. 2015. Patients with irritable bowel syndrome-diarrhea have lower disease-specific quality of life than irritable bowel syndrome-constipation. *World J Gastroenterol,* 21, 8103-9.

Sisson, G., Ayis, S., Sherwood, R. A. & Bjarnason, I. 2014. Randomised clinical trial: A liquid multi-strain probiotic vs. placebo in the irritable bowel syndrome--a 12 week double-blind study. *Aliment Pharmacol Ther,* 40, 51-62.

Smith, C. J. & Osborn, A. M. 2009. Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. *FEMS Microbiol Ecol,* 67, 6-20.

Sood, R., Gracie, D. J., Law, G. R. & Ford, A. C. 2015. Systematic review with meta-analysis: the accuracy of diagnosing irritable bowel syndrome with symptoms, biomarkers and/or psychological markers. *Aliment Pharmacol Ther*. 42(5), 491-503.

Spiegel, B., Camilleri, M., Bolus, R., Andresen, V., Chey, W. D., Fehnel, S., Mangel, A., Talley, N. J. & Whitehead, W. E. 2009. Psychometric evaluation of patient-reported outcomes in irritable bowel syndrome randomized controlled trials: a Rome Foundation report. *Gastroenterology,* 137, 1944-53.

Spiegel, B. M., Bolus, R., Agarwal, N., Sayuk, G., Harris, L. A., Lucak, S., Esrailian, E., Chey, W. D., Lembo, A., Karsan, H., Tillisch, K., Talley, J. & Chang, L. 2010a. Measuring symptoms in

the irritable bowel syndrome: development of a framework for clinical trials. *Aliment Pharmacol Ther,* 32, 1275-91.

Spiegel, B. M., Bolus, R., Harris, L. A., Lucak, S., Chey, W. D., Sayuk, G., Esrailian, E., Lembo, A., Karsan, H., Tillisch, K., Talley, J. & Chang, L. 2010b. Characterizing abdominal pain in IBS: guidance for study inclusion criteria, outcome measurement and clinical practice. *Aliment Pharmacol Ther,* 32, 1192-202.

Spiegel, B. M., Gralnek, I. M., Bolus, R., Chang, L., Dulai, G. S., Mayer, E. A. & Naliboff, B. 2004. Clinical determinants of health-related quality of life in patients with irritable bowel syndrome. *Arch of Intern Med,* 164, 1773-80.

Spiller, R. 2007. Recent advances in understanding the role of serotonin in gastrointestinal motility in functional bowel disorders: alterations in 5-HT signalling and metabolism in human disease. *Neurogastroenterol Motil,* 19 Suppl 2, 25-31.

Spiller, R., Aziz, Q., Creed, F., Emmanuel, A., Houghton, L., Hungin, P., Jones, R., Kumar, D., Rubin, G., Trudgill, N. & Whorwell, P. 2007. Guidelines on the irritable bowel syndrome: mechanisms and practical management. *Gut,* 56, 1770-98.

Squires, R. W. & Hartsell, S. E. 1955. Survival and growth initiation of defrosted Escherichia coli as affected by frozen storage menstrua. *Appl Microbiol,* 3, 40-5.

Staudacher, H. M., Irving, P. M., Lomer, M. C. & Whelan, K. 2014. Mechanisms and efficacy of dietary FODMAP restriction in IBS. *Nat Rev Gastroenterol Hepatol,* 11, 256-66.

Staudacher, H. M., Lomer, M. C., Anderson, J. L., Barrett, J. S., Muir, J. G., Irving, P. M. & Whelan, K. 2012. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *J Nutr,* 142, 1510-8.

Staudacher, H. M., Whelan, K., Irving, P. M. & Lomer, M. C. 2011. Comparison of symptom response following advice for a diet low in fermentable carbohydrates (FODMAPs) versus standard dietary advice in patients with irritable bowel syndrome. *J Hum Nutr Diet,* 24, 487-95.

Sterne, J. A., White, I. R., Carlin, J. B., Spratt, M., Royston, P., Kenward, M. G., Wood, A. M. & Carpenter, J. R. 2009. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ,* 338, b2393.

Storro, O., Avershina, E. & Rudi, K. 2013. Diversity of intestinal microbiota in infancy and the risk of allergic disease in childhood. *Curr Opin Allergy Clin Immunol,* 13, 257-62.

Suarez, F. L., Springfield, J., Furne, J. K., Lohrmann, T. T., Kerr, P. S. & Levitt, M. D. 1999. Gas production in human ingesting a soybean flour derived from beans naturally low in oligosaccharides. *Am J Clin Nutr,* 69, 135-9.

Sundin, J., Rangel, I., Fuentes, S., Heikamp-de Jong, I., Hultgren-Hornquist, E., de Vos, W. M. & Brummer, R. J. 2015. Altered faecal and mucosal microbial composition in post-infectious irritable bowel syndrome patients correlates with mucosal lymphocyte phenotypes and psychological distress. *Aliment Pharmacol Ther,* 41, 342-51.

Svedlund, J., Sjodin, I. & Dotevall, G. 1988. GSRS--a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci,* 33, 129-34.

Tan, S. C. & Yiap, B. C. 2009. DNA, RNA, and protein extraction: the past and the present. *J Biomed Biotechnol,* 2009, 574398.

Tana, C., Umesaki, Y., Imaoka, A., Handa, T., Kanazawa, M. & Fukudo, S. 2010. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol Motil,* 22, 512-9.

Tap, J., Furet, J. P., Bensaada, M., Philippe, C., Roth, H., Rabot, S., Lakhdari, O., Lombard, V., Henrissat, B., Corthier, G., Fontaine, E., Dore, J. & Leclerc, M. 2015. Gut microbiota

richness promotes its stability upon increased dietary fibre intake in healthy adults. *Environ Microbiol*, Epub ahead of print.

Taylor, S., Wakem, M., Dijkman, G., Alsarraj, M. & Nguyen, M. 2010. A practical approach to RT-qPCR-Publishing data that conform to the MIQE guidelines. *Methods,* 50, S1-5.

Thabane, L., Mbuagbaw, L., Zhang, S., Samaan, Z., Marcucci, M., Ye, C., Thabane, M., Giangregorio, L., Dennis, B., Kosa, D., Borg Debono, V., Dillenburg, R., Fruci, V., Bawor, M., Lee, J., Wells, G.*, et al.* 2013. A tutorial on sensitivity analyses in clinical trials: the what, why, when and how. *BMC Med Res Methodol,* 13, 92.

The Human Microbiome Project Consortium 2012. Structure, function and diversity of the healthy human microbiome. *Nature,* 486, 207-14.

Theoharides, T. C. 2014. Mast cells in irritable bowel syndrome and ulcerative colitis: function not numbers is what makes all the difference. *Dig Dis Sci,* 59, 897-8.

Thompson, W. G., Heaton, K. W., Smyth, G. T. & Smyth, C. 2000. Irritable bowel syndrome in general practice: prevalence, characteristics, and referral. *Gut,* 46, 78-82.

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., Guyonnet, D., Legrain–Raspaud, S., Trotin, B., Naliboff, B. & Mayer, E. A. 2013. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology,* 144(7), 1394-401.

Tillisch, K. & Labus, J. S. 2011. Advances in imaging the brain-gut axis: functional gastrointestinal disorders. *Gastroenterology,* 140, 407-411.

Topping, D. L. & Clifton, P. M. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev,* 81, 1031-64.

Tornblom, H., Van Oudenhove, L., Tack, J. & Simren, M. 2014. Interaction between preprandial and postprandial rectal sensory and motor abnormalities in IBS. *Gut,* 63, 1441-9.

Trentacosti, A. M., He, R., Burke, L. B., Griebel, D. & Kennedy, D. L. 2010. Evolution of clinical trials for irritable bowel syndrome: issues in end points and study design. *Am J Gastroenterol,* 105, 731-5.

Truswell, A. S., Seach, J. M. & Thorburn, A. W. 1988. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. *Am J Clin Nutr,* 48, 1424-30.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R. & Gordon, J. I. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature,* 444, 1027-31.

van Loo, J. C., P.de, Leenheer L.Hoebregs, H.Smits, G. 1995. On the presence of inulin and oligofructose as natural ingredients in the western diet. *Crit Rev Food Sci Nutr,* 35, 525-552.

Vanhoutvin, S. A., Troost, F. J., Kilkens, T. O., Lindsey, P. J., Hamer, H. M., Jonkers, D. M., Venema, K. & Brummer, R. J. 2009. The effects of butyrate enemas on visceral perception in healthy volunteers. *Neurogastroenterol Motil,* 21, 952-e76.

Verdu, E. F., Bercik, P., Bergonzelli, G. E., Huang, X. X., Blennerhasset, P., Rochat, F., Fiaux, M., Mansourian, R., Corthesy-Theulaz, I. & Collins, S. M. 2004. Lactobacillus paracasei normalizes muscle hypercontractility in a murine model of postinfective gut dysfunction. *Gastroenterology,* 127, 826-37.

Villani, A. C., Lemire, M., Thabane, M., Belisle, A., Geneau, G., Garg, A. X., Clark, W. F., Moayyedi, P., Collins, S. M., Franchimont, D. & Marshall, J. K. 2010. Genetic risk factors for post-infectious irritable bowel syndrome following a waterborne outbreak of gastroenteritis. *Gastroenterology,* 138, 1502-13.

Walker, A. W., Ince, J., Duncan, S. H., Webster, L. M., Holtrop, G., Ze, X., Brown, D., Stares, M. D., Scott, P., Bergerat, A., Louis, P., McIntosh, F., Johnstone, A. M., Lobley, G. E., Parkhill, J. & Flint, H. J. 2011. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J,* 5, 220-30.

Walter, J. & Ley, R. 2011. The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol,* 65, 411-29.

Ware, J. E., Jr. & Sherbourne, C. D. 1992. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care,* 30, 473-83.

Watson, B. W., Meldrum, S. J., Riddle, H. C., Brown, R. L. & Sladen, G. E. 1972. pH profile of gut as measured by radiotelemetry capsule. *BMJ,* 2, 104-6.

Whelan, K. 2014. Editorial: The importance of systematic reviews and meta-analyses of probiotics and prebiotics. *Am J Gastroenterol,* 109, 1563-5.

Whelan, K., Abrahmsohn, O., David, G. J., Staudacher, H., Irving, P., Lomer, M. C. & Ellis, P. R. 2011. Fructan content of commonly consumed wheat, rye and gluten-free breads. *Int J Food Sci Nutr,* 62, 498-503.

Whelan, K., Judd, P. A., Preedy, V. R., Simmering, R., Jann, A. & Taylor, M. A. 2005. Fructooligosaccharides and fiber partially prevent the alterations in fecal microbiota and short-chain fatty acid concentrations caused by standard enteral formula in healthy humans. *J Nutr,* 135, 1896-1902.

Whelan, K., Judd, P. A. & Taylor, M. A. 2003. Defining and reporting diarrhoea during enteral tube feeding: do health professionals agree? *J Hum Nutr Diet,* 16, 21-6.

Whitehead, W. E., Palsson, O. S., Levy, R. L., Feld, A. D., VonKorff, M. & Turner, M. 2006. Reports of "satisfactory relief" by IBS patients receiving usual medical care are confounded by baseline symptom severity and do not accurately reflect symptom improvement. *Am J of Gastroenterol,* 101, 1057-65.

Whitehead, W. E., Palsson, O. S., Levy, R. R., Feld, A. D., Turner, M. & Von Korff, M. 2007. Comorbidity in irritable bowel syndrome. *Am J Gastroenterol,* 102, 2767-76.

Whorwell, P. J., Altringer, L., Morel, J., Bond, Y., Charbonneau, D., O'Mahony, L., Kiely, B., Shanahan, F. & Quigley, E. M. 2006. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol,* 101, 1581-90.

Wiklund, I. K., Fullerton, S., Hawkey, C. J., Jones, R. H., Longstreth, G. F., Mayer, E. A., Peacock, R. A., Wilson, I. K. & Naesdal, J. 2003. An irritable bowel syndrome-specific symptom questionnaire: development and validation. *Scand J Gastroenterol,* 38, 947-54.

Wilder-Smith, C. H., Materna, A., Wermelinger, C. & Schuler, J. 2013. Fructose and lactose intolerance and malabsorption testing: the relationship with symptoms in functional gastrointestinal disorders. *Aliment Pharmacol Ther,* 37, 1074-83.

Williams, E. A., Nai, X. & Corfe, B. M. 2011. Dietary intakes in people with irritable bowel syndrome. *BMC Gastroenterol,* 11, 9.

Williams, M. D., Ha, C. Y. & Ciorba, M. A. 2010. Probiotics as therapy in gastroenterology: a study of physician opinions and recommendations. *J Clin Gastroenterol,* 44, 631-6.

Williams, R. E., Black, C. L., Kim, H. Y., Andrews, E. B., Mangel, A. W., Buda, J. J. & Cook, S. F. 2006. Determinants of healthcare-seeking behaviour among subjects with irritable bowel syndrome. *Aliment Pharmacol Ther,* 23, 1667-75.

Windey, K., De Preter, V., Huys, G., Broekaert, W. F., Delcour, J. A., Louat, T., Herman, J. & Verbeke, K. 2014. Wheat bran extract alters colonic fermentation and microbial composition, but does not affect faecal water toxicity: a randomised controlled trial in healthy subjects. *Br J Nutr*, 12, 1-14.

Windhauser, M. M., Evans, M. A., McCullough, M. L., Swain, J. F., Lin, P. H., Hoben, K. P., Plaisted, C. S., Karanja, N. M. & Vollmer, W. M. 1999. Dietary adherence in the Dietary Approaches to Stop Hypertension trial. DASH Collaborative Research Group. *J Am Diet Assoc,* 99, S76-83.

Wong, R. K. & Drossman, D. A. 2010. Quality of life measures in irritable bowel syndrome. *Expert Rev Gastroenterol Hepatol,* 4, 277-84.

Wong, R. K., Yang, C., Song, G. H., Wong, J. & Ho, K. Y. 2015. Melatonin regulation as a possible mechanism for probiotic (VSL#3) in irritable bowel syndrome: a randomized double-blinded placebo study. *Dig Dis Sci,* 60, 186-94.

Wood, J., Scott, K. P., Avgustin, G., Newbold, C. J. & Flint, H. J. 1998. Estimation of the relative abundance of different Bacteroides and Prevotella ribotypes in gut samples by restriction enzyme profiling of PCR-amplified 16S rRNA gene sequences. *Appl Environ Microbiol,* 64, 3683-9.

World Health Organisation 2009. Irritable bowel syndrome: a global perspective. Retrieved from: http://www.worldgastroenterology.org/guidelines/global-guidelines/irritable-bowel-syndrome-ibs.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H*., et al.* 2011. Linking long-term dietary patterns with gut microbial enterotypes. *Science,* 334, 105-8.

Wyrwich, K. W. & Tardino, V. M. 2004. A blueprint for symptom scales and responses: measurement and reporting. *Gut,* 53, iv45-iv8.

Yang, J., Den, Y., Chiu, H., Cong,Y., Zhao, J., Pohl, D., Misselwitz, B., Fried, M., Dai, N. & Fox, M. 2013. Prevalnce and presenation of lactose intolerance and effects on daity product intake in healthy subjects and patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol*, 11, 262-8.

Yao, C. K., Gibson, P. R. & Shepherd, S. J. 2013. Design of clinical trials evaluating dietary interventions in patients with functional gastrointestinal disorders. *Am J Gastroenterol,* 108, 748-58.

Yao, C. K., Tan, H. L., van Langenberg, D. R., Barrett, J. S., Rose, R., Liels, K., Gibson, P. R. & Muir, J. G. 2014. Dietary sorbitol and mannitol: food content and distinct absorption patterns between healthy individuals and patients with irritable bowel syndrome. *J Hum Nutr Diet,* 27 Suppl 2, 263-75.

Yao, X., Yang, Y. S., Cui, L. H., Zhao, K. B., Zhang, Z. H., Peng, L. H., Guo, X., Sun, G., Shang, J., Wang, W. F., Feng, J. & Huang, Q. 2012. Subtypes of irritable bowel syndrome on Rome III criteria: a multicenter study. *J Gastroenterol Hepatol,* 27, 760-5.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A*., et al.* 2012. Human gut microbiome viewed across age and geography. *Nature,* 486, 222-7.

Young, S. L., Blanco, I., Hernandez-Cordero, S., Pelto, G. H. & Neufeld, L. M. 2010. Organoleptic properties, ease of use, and perceived health effects are determinants of acceptability of micronutrient supplements among poor Mexican women. *J Nutr,* 140, 605-11.

Zaki, R., Bulgiba, A., Ismail, R. & Ismail, N. A. 2012. Statistical methods used to test for agreement of medical instruments measuring continuous variables in method comparison studies: a systematic review. *PLoS One,* 7, e37908.

Zeng, J., Li, Y. Q., Zuo, X. L., Zhen, Y. B., Yang, J. & Liu, C. H. 2008. Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther,* 28, 994-1002.

Zhou, Q. & Verne, G. N. 2011. New insights into visceral hypersensitivity--clinical implications in IBS. *Nat Rev Gastroenterol Hepatol,* 8, 349-55.

Zhou, Q., Zhang, B. & Verne, G. N. 2009. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. *Pain,* 146, 41-6.

Zhu, Y., Zheng, X., Cong, Y., Chu, H., Fried, M., Dai, N. & Fox, M. 2013. Bloating and distention in irritable bowel syndrome: the role of gas production and visceral sensation after

lactose ingestion in a population with lactase deficiency. *Am J Gastroenterol,* 108, 1516-25.

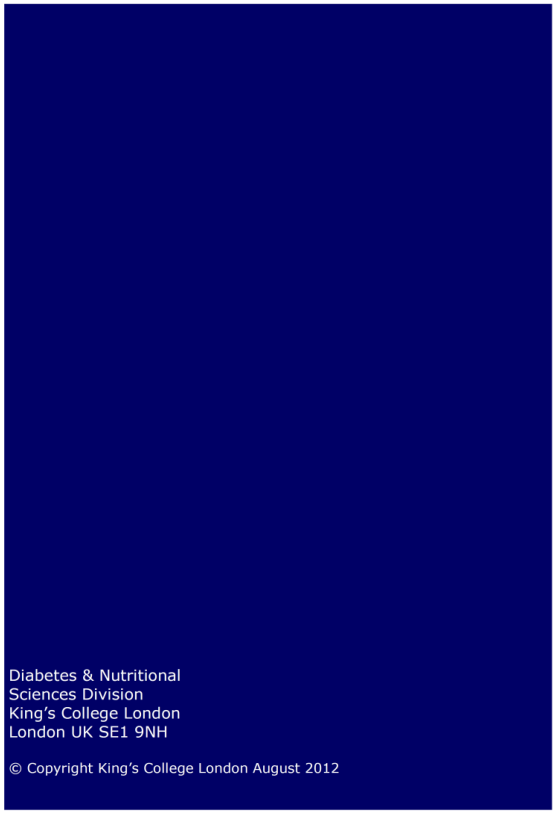
Zoetendal, E. G., Collier, C. T., Koike, S., Mackie, R. I. & Gaskins, H. R. 2004. Molecular ecological analysis of the gastrointestinal microbiota: a review. *J Nutr,* 134, 465-72.

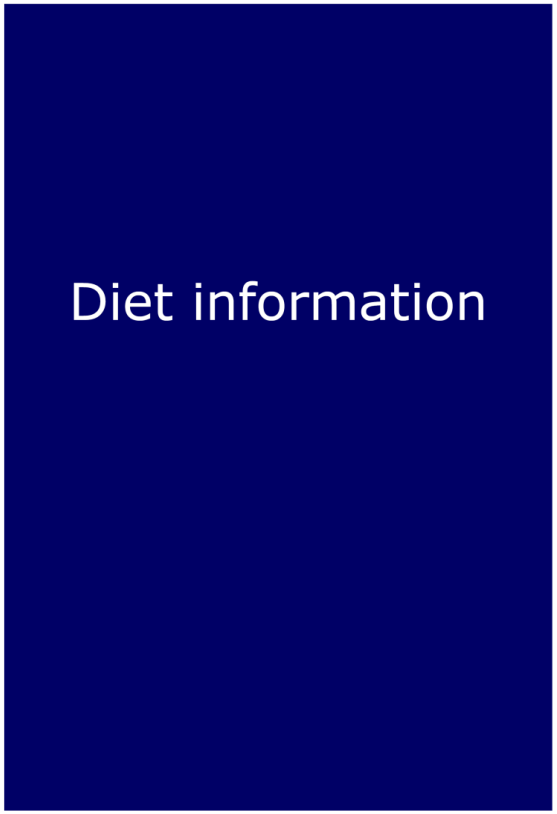
Zoetendal, E. G., von Wright, A., Vilpponen-Salmela, T., Ben-Amor, K., Akkermans, A. D. & de Vos, W. M. 2002. Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Appl Environ Microbiol,* 68, 3401-7.

9 Appendices

9.1 Written dietary resource sample pages

Low FODMAP resource





Suitable foods: starchy foods

Aim to include starchy food at each meal. 1 portion is 3 tbs breakfast cereal or porridge or 1 slice bread or 3 crackers or 2 small potatoes or 2 tbs cooked rice or pasta

Cereal grains & starchy foods
Rice, potato, sweet potato, oats, oat bran, buckwheat, polenta, quinoa

Bread
Wheat free or gluten free bread and rolls (white and fibre)
Spelt bread
Bread made from oat, rice, corn, potato flours
Wheat free or gluten free pizza bases, wheat free or gluten free pitta bread, wheat free or gluten free ciabatta, wheat free or gluten free naan bread
Homemade wheat free bread using a breadmaker is a good option for fresh bread every day

Flour and raising agents
Wheat free or gluten free flour, arrowroot, buckwheat, cornflour, maize flour, polenta, potato flour, rice flour
Baking powder, bicarbonate of soda, cream of tartar, yeast

Pasta Wheat free or gluten free pasta, buckwheat pasta

Noodles
Rice noodles, buckwheat noodles

Breakfast cereals
Porridge and oat based cereals, cornflakes, rice krispies. *Check ingredients label for problem fruit, FOS, inulin, oligofructose*

Savoury biscuits and snacks
Rice crackers, corncakes, oatcakes
Popcorn
Plain or ready salted crisps
Check ingredients label for fructose and sorbitol

Sweet biscuits and cakes
Macaroons, oat based biscuits, flapjack. Most 'free from' varieties are wheat free
Flourless cakes, meringues, cornflour sponge
Check ingredients label for fructose and sorbitol

Pastry
Wheat free or gluten free varieties and mixes

Breadcrumbs
Polenta, oats, cornflake crumbs,

Foods to avoid: starchy foods

Cereal grains
Wheat (including bulghur wheat, couscous, semolina)
Rye, barley, amaranth

Bread
All wheat bread and rolls (white, wholemeal, multigrain, sourdough, rye bread)
Pitta bread, ciabatta, focaccia, panini, naan bread, chapatti, croissants, muffins, brioche, pastries
Garlic bread, pizza

Flour
All wheat flour (white, wholemeal, plain, self-raising)
Rye flour, barley flour

Pasta
All fresh and dried pasta (white, wholemeal), spelt pasta
Gnocchi

Noodles
Egg noodles, Hokkein, Udon
Pot noodles, Supernoodles, Ramen

Breakfast cereals
Wheat or bran based cereals (Weetabix, Shredded Wheat, bran flakes, All-bran, Cheerios, muesli) wheat bran, wheat germ

Savoury biscuits and snacks
Water biscuits, crispbreads, rye crispbreads, wheat crackers, cream crackers, spelt crackers

Sweet biscuits and cakes
All biscuits and cakes made with wheat flour (digestives, shortbread, rich tea, custard creams, cookies, fruit cake, fairy cakes, Victoria sponge, chocolate cake)

Pastry
All pastry made with wheat flour (shortcrust, puff, flaky, filo)
Shop bought pastry and pastry goods (pies, quiche, pasties)

Breadcrumbs and batter
Crumbed fish and poultry, fish fingers, fish in batter, tempura batter, Scotch eggs

Minor wheat, rye or barley ingredients do not need to be avoided (thickeners, starches and flavourings)

Sham diet resource

Diabetes & Nutritional
Sciences Division
King's College London
London UK SE1 9NH

Diet information

Suitable foods: starchy foods

Aim to include starchy food at each meal. 1 portion is 3 tbs breakfast cereal or 1 slice bread or 3 crackers or 2 small potatoes or 2 tbs cooked pasta

Cereal grains & other starchy foods
Wheat (e.g. cous cous, bulghur wheat, breads and breakfast cereals listed), potato, sweet potato, polenta, quinoa, barley, amaranth

Bread
All wheat bread and rolls
White, wholemeal, multigrain, sourdough breads
Cornbread
Pitta bread, ciabatta, focaccia, panini, naan bread, chapatti, croissants, muffins, brioche, pastries, crumpets, English muffins, garlic bread, pizza

Flour and raising agents
Wheat flour (white, wholemeal, plain, self-raising)
Baking power, bicarbonate of soda, cream of tartar, yeast

Breakfast cereals
Wheat or bran based cereals (Weetabix, Shredded Wheat, bran flakes, All-bran, Cheerios, Fruit and Fibre)
Cornflakes, cornmeal porridge

Pasta
Fresh and dried wheat pasta (white, wholemeal), gnocchi

Noodles
Egg, Hokkein, Udon, Ramen, pot noodles, Supernoodles

Savoury biscuits and snacks
Water biscuits, crispreads, wheat crackers, cream crackers, corn cakes
Popcorn

Sweet biscuits and cakes
Digestives, shortbread, rich tea, custard creams, cookies, fruit cake, fairy cakes, Victoria sponge, chocolate cake

Pastry
All pastry (shortcrust, puff, flaky, filo)
Shop bought pastry and pastry goods (pies, quiche, pasties)

Breadcrumbs
Crumbed fish and poultry, fish fingers, fish in batter, tempura batter, Scotch eggs

Foods to avoid: starchy foods

Cereal grains
Rice
Oats
Rye
Spelt
Buckwheat
Millet

Bread
Rice bread
Spelt bread
Rye bread, black bread
Soda bread

Flour
Rice flour
Rye flour
Spelt flour
Buckwheat flour
Millet flour
(minor amounts do not need to be avoided)

Breakfast cereals
Rice Krispies and rice cereals
Porridge oats and oat cereals
Cereals and muesli containing oat, rye, spelt, buckwheat or millet
Bircher muesli
Cereal, muesli or granola containing unsuitable fruits
Buckwheat porridge
Buckwheat pancakes (galettes)

Pasta
Rye pasta
Spelt pasta
Buckwheat pasta
Millet pasta

Noodles
Rice noodles
Buckwheat (soba) noodles

Savoury biscuits and snacks
Rice cakes and Snack A Jacks
Oatcakes
Rye crispbreads
Spelt crackers
Crackers containing buckwheat or millet

Sweet biscuits and cakes
Flapjack
Biscuits or cakes made with oats, rice flour, spelt, buckwheat or millet
Cakes or biscuits containing unsuitable fruit

Pastry
Pies, pastries containing unsuitable fruit

Rice based meals
Sushi
Fried rice
Risotto
Paella

Guy's and St Thomas' NHS
NHS Foundation Trust
Chief Investigator: Dr Kevin Whelan
Principal Investigator: Dr Peter Irving & Heidi Staudacher

KING'S College LONDON

9.2 Participant information sheet

Participant Information Sheet

The impact of dietary interventions for irritable bowel syndrome on luminal microbiota, symptoms, nutrient intake and quality of life: a randomised controlled trial

We would like to invite you to take part in a research study conducted by King's College London and Guy's and St Thomas' Hospital to assess the effect of dietary interventions in irritable bowel syndrome (IBS). Before you decide we would like you to understand why the research is being done and what it would involve for you. We will go through this information sheet and answer any questions you have.

What is the purpose of the study?

The purpose of this 5-week study is to investigate the effect of dietary interventions on the bacteria in your bowel. One diet intervention that alters some carbohydrates in your diet (e.g. the types of fruits and vegetables) might be effective for symptoms such as bloating, abdominal pain and flatulence for many people with IBS.

There are certain bacteria in your bowel e.g. bifidobacteria. Recent research has shown that the diet intervention described above can impact on the concentration of bifidobacteria in your bowel.

Probiotics are friendly bacteria added to foods that can increase bifidobacteria in the bowel. This study will investigate the effect of the dietary intervention with a probiotic food supplement on:

- Bacteria in the bowel and products of bacterial fermentation
- Gut behaviour (e.g. wind, bloating)
- Stool frequency and consistency
- Dietary intake
- Quality of life

Who are we recruiting?

We are looking for approximately 106 men and women between 18 and 65 years of age who have IBS but without constipation as the predominant symptom. People who have other gut conditions or who have diabetes, psychiatric or other chronic diseases will not be eligible to take part. People who have recently taken certain medications (e.g. prebiotics, probiotics or antibiotics) will not be eligible. Pregnant or breastfeeding women will also be excluded.

Do I have to take part?

It is up to you to decide whether to take part in the study. We will describe the study and go through this information sheet with you. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason.

What will happen to me if I take part?

The study will last for five weeks, and you will attend two or three visits at King's College London throughout the study to provide samples and have measurements taken. Each visit should last no more than 1 hour. Further details about each visit can be found in this information sheet.

What will I have to do?

Screening visit:

If you are interested in taking part we will fully explain all study procedures and check that you are eligible. The consent form will need to be signed prior to the start of the study and this may involve an additional visit to the study site. Once the consent form is signed, we need to check your symptoms are severe enough and so we will need you to record information about your gut symptoms and your bowel habits each day for 7 days, and also record all you eat and drink for 7 days. These diaries should take no more than a total of 1 hour to complete spread over the 7 days and if you take part this will form the baseline data for the study.

Visit 1 and 2:

At the end of the 7-day baseline period you will attend King's College London for Visit 1 and four weeks later you will attend Visit 2.

At these visits:

- We will review your symptom/stool diary and diet diary
- We will record your medical and medication history and smoking status (visit 1 only)
- We will check your weight
- We will check your height (visit 1 only)
- You will provide a fresh stool sample. You will need to provide the stool sample using a stool collection kit so that you can collect the whole stool.
- You will be asked to complete an IBS symptom questionnaire, two quality of life questionnaires and an acceptability questionnaire (these will take no more than 35 minutes)

Interventions

At Visit 1 you will be randomly assigned one of the following four groups. You will not know which group you are in.

Group 1: dietary intervention + probiotic food supplement
Group 2: dietary intervention + placebo food supplement
Group 3: sham diet + probiotic food supplement
Group 4: sham diet + placebo food supplement

The dietary intervention is the diet that might help people with IBS and the 'sham' diet is the control diet. The control diet is used as a comparison and has been devised to appear like a diet to help with your IBS. A specialised dietitian who is part of the research team will provide the dietary advice and give you detailed written information about the diet you need to follow.

The probiotic food supplement is a tasteless commercially available powder supplement that you will add to your diet daily. A placebo is a dummy treatment, which looks like the real thing but is not. It contains no active ingredient. The researcher who gives you the probiotic or placebo will also not know which one you are receiving. You will need to take the probiotic/placebo twice a day. It doesn't matter when you take the supplement as long as you take two a day. You will be given a compliance diary to complete to help us check how often you took the probiotic or placebo.

A member of the research team will call you on a weekly basis during the 4 weeks to answer any questions and to remind you when to start your diaries.

Throughout the study, we will ask that you do not consume any live (probiotic) yoghurts or prebiotic supplements (see pages 6,7). In addition, should it be medically necessary for you to take antibiotics, you will be withdrawn from the study. Should this occur please contact the research team as soon as possible.

Alternative treatments

Your doctor will discuss alternative treatments for your condition, which includes medications or other dietary treatments offered at Guy's and St Thomas' Hospital.

Expenses

Reimbursements for expenses (e.g. travel, meals, child-care, compensation for loss of earnings, etc.) will not be available for this study. No special arrangements will be made for compensation because this diet is routine treatment for people with IBS.

What are the possible disadvantages and risks of taking part?

An ethical review of this study has been carried out. The diet and probiotic food supplement have no known adverse effects and have been well tolerated in previous studies.

Some people might find recording symptoms and/or collecting stool as part of the study embarrassing. You will be provided a toilet insert in which to collect your sample which will make this process easier. If you are concerned in any way please contact one of the researchers for advice.

What are the possible benefits of taking part?

The results of this study may help to answer scientific questions about what happens in your bowel when following the diet under investigation in conjunction with a probiotic food supplement, therefore helping other people in the future. In addition, it is possible that the study may result in improvements in your IBS symptoms. However, it is possible that no therapeutic or direct health benefits may result during or following your participation in this study.

What if new information becomes available?

If additional information becomes available during the course of the research regarding the diet or probiotic food supplement that is being studied, we will speak with you about it and whether you want to continue in the study. If you decide to withdraw we will make arrangements for your care to continue. If you decide to continue you may be asked to sign an updated consent form. Also, on receiving this new information the research team might consider it in your best interests to withdraw from the study. We will explain the reasons and arrange for your care to continue.

What happens at the end of the study?

At the end of the 5-week study we will tell you whether you received the intervention or the sham diet. If you received the intervention diet we will provide you with advice regarding the reintroduction of food items back into your diet just like we would in routine clinical care. If you received the sham diet and wish to receive advice regarding the dietary intervention we are investigating we can provide this at Visit 2 and then book you into a dietetic outpatient appointment for follow-up if you wish. You can also return to your usual diet if you would like. If you would like to know the probiotic food supplement that was being investigated we will tell you the product name at Visit 2 in order that you can purchase it if you wish.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to Heidi Staudacher or one of the researchers who will do their best to answer your questions (**0207 848 4447**). We would not expect you to suffer any harm or injury because of your participation in this study. However, in the unlikely event of you suffering any adverse effects as a consequence of participating in this study you can contact King's College London using the details below for further advice and information and may be compensated through King's College London's 'No Fault Compensation Scheme'. Dr Kevin Whelan, Senior Lecturer in Nutritional Sciences, Tel: 020 78 48 38 58,
Email: kevin.whelan@kcl.ac.uk

Will my taking part in the study be kept confidential?

We will contact your local doctor to inform them of your participation in the study. Any information/samples that leave King's College London will have your name, date of birth and hospital number removed so that you cannot be recognised from them. Data will be stored securely and only the research team will have access. Part of your medical records and information obtained during the study may be read by regulatory authorities to confirm the data collected. Your personal information will be strictly confidential and will not be made publicly available. If the results of the study are published, your identity will continue to remain confidential. Personal data and unidentifiable research data will be kept for 10 years.

What will happen if I don't want to carry on with the study?

Taking part in this study is voluntary and your decision will in no way affect your current or future care within this trust. You will not lose any of your legal or ethical rights. You may withdraw from the study at any time without affecting your routine clinical care and you are not obliged to give reasons. However, if you withdraw because of a side effect please inform the research team. You may be withdrawn from the study, if it is considered in your best interests. If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

What will happen to any samples I give?

During the study we will collect stool samples. The samples will have no personal details on them, so your identity will not be recognisable. Portions of the samples may be stored in a secure freezer for up to 10 years. Only members of the research team will have access to them. After they have been used, or at the end of 10 years they will be destroyed.

What will happen to the results of the research study?

It is intended that the results of this study may be published in scientific or medical journals. You will not be identified in any report or publication. When the data from the study has been analysed you will receive a summary report of the results.

Who has reviewed the study?

This study has been checked by an independent group of people, called a Research Ethics Committee to protect your safety, rights, well-being and dignity. This study has been reviewed and given favourable opinion by the NRES Committee London - Fulham.

Who is organising and funding the research?

The study is organised and sponsored by King's College London and funded by internal funding (Diabetes and Nutritional Sciences Division, King's College London). The Principal Investigator is Heidi Staudacher. The Chief Investigator is Dr Kevin Whelan

Further information and contact details

If you would like further information about this research please contact Heidi Staudacher on **0207 848 4447**.

Eating and drinking during the study

Before the study it is **important that you to continue to follow your usual diet**, eating and drinking as you normally would.

Before and during the study it is also important that you avoid foods, drinks and supplements that contain probiotics or prebiotics. This is because probiotics and prebiotics affect the bacteria in your gut – one of the things we are measuring.

Probiotics and prebiotics are mostly found in yoghurts, yoghurt drinks and supplements. However, they can be found in other foods such as smoothies, cereals and cheese. You can usually tell from the label if there's a prebiotic or probiotic in a food or supplement. These labels will usually contain the following words:

- Prebiotic
- Probiotic
- Bio
- Live culture
- Lactic cultures
- Yogurt cultures
- Lactobacillus
- Acidophilus
- L. acidophilus
- Fructooligosaccharide
- Oligofructose
- Inulin

Yogurt
Most yogurts contain probiotic, including many supermarket own brands. If you eat yogurt please check the label for the words listed above and select an alternative from the products listed below.

Yogurts to avoid	**Suitable alternatives**
Alpro (soya yogurt) Activia Benecol Bon Maman Muller Vitality Onken Rachel's Shape Ski Total Yeo Valley Weight Watchers	Müller corners (except Breakfast variety) Müller Light Müller Amoré Nom Yoplait Yop Drinking Yoghurt Yoplait Perle de Lait Frozen yoghurt e.g. Sainsbury's or Yeo Valley Fromage frais (e.g. Petit Filous) is usually not probiotic and can be used instead of yogurt
Own brand, sheep or goat's milk yoghurt or other yogurts labeled with any of the words listed above.	Own brand or other yogurts where the words listed above do NOT appear on the label or in the ingredients list. .

Other foods, drinks and supplements
Below is a list of other products that can be found in supermarkets and health food shops that report that they contain prebiotics or probiotics. (This list is not comprehensive – other products may contain prebiotics and/or probiotics).

Please avoid the products listed below during the study. If you are unsure about a particular product please contact us to discuss it.

- Alpen Light Cereal Bars
- ASDA Cholesterol Lowering Yoghurt Drinks
- ASDA Inner Defence Yoghurt Drinks
- ASDA Vitality Cereal Bars
- Bassetts Soft & Chewy Active Health Multivitamins With Minerals
- Benecol Drinking Yoghurt
- Biomuno products
- Boots Probiotic Multivitamins
- Boots Biobalance Support
- Danone Actimel Yoghurt Drinks
- Flora Pro Activ Cholesterol Lowering Health Drinks
- Healthspan Probiotic Capsules
- Healthspan Regulease
- Healthspan Super20 Probiotic
- Holland And Barrett Acidophilus Tablets
- Kellogg's All Bran Breakfast Biscuits
- Kellogg's Fibre Plus Cereal Bars
- Moma Oat Breakfast Products
- Muller Vitality Yoghurt Drinks
- Multibionta Probiotic Multivitamins
- Probio 7 Pre Or Probiotics
- Quaker Morning Bars
- Rice Krispies Multigrain
- Sainsbury's Fruit Of The Forest Yoghurt Slices
- Sainsbury's Probiotic Be Good To Yourself Yoghurt Drinks
- Tesco Fruit And Fibre Cereal Bars
- Tesco Light Choices Cereal Bars
- Tesco Probiotic Yoghurt Drinks
- VSL #3
- Weetabix Oaty Bars
- Weetabix Oatibix Sultana And Apple Bitesize Cereal
- Yakult

9.3 Probiotic/placebo compliance diary

COMPLIANCE DIARY: WEEK 1

Week commences date: _____

Please record every time you take the probiotic/placebo food supplement

Day	Record 'x'	
1	1 ☐	
	2 ☐	
2	1 ☐	
	2 ☐	
3	1 ☐	
	2 ☐	
4	1 ☐	
	2 ☐	
5	1 ☐	
	2 ☐	
6	1 ☐	
	2 ☐	
7	1 ☐	**The researcher will call you today**
	2 ☐	

Guy's and St Thomas' NHS
NHS Foundation Trust

KING'S College LONDON

CONSENT FORM

9.4 **Consent form**

Title of project: The impact of dietary interventions for irritable bowel syndrome on luminal microbiota, symptoms, nutrient intake and quality of life: a randomised controlled trial

Participant ID:		**Please initial**
1.	I consent to the following: • Two visits at King's College London • Taking part in a four week dietary intervention • Take a probiotic food supplement or placebo supplement daily • Complete questionnaires and diaries as described in the participant information sheet	
2.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3.	I agree that my stool samples can be used in the above study and I have been made aware of how any surplus material will be disposed of.	
4.	I agree that any data, blood and stool samples that are surplus to this study, as well as any relevant information, can be used in future research that has been approved by a recognised Research Ethics Committee, but that my identity will be kept anonymous.	
5.	I consent to the processing of my personal information for the purposes explained to me. I understand that such information will be handled in accordance with the terms of the Data Protection Act 1998.	
6.	I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from Guy's and St Thomas' NHS Foundation Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
7.	I agree to my GP being informed of my participation in the study.	
8.	I agree to take part in the above study.	

Name of Participant	Date	Signature
Name of person taking consent (if different from Investigator)	Date	Signature
Investigator	Date	Signature

9.5 Symptom, stool and diet record

Participant Diary
Baseline (example only)

Screening ID

Randomisation ID

SECTION ONE

Introduction

This is your Diary Booklet for **Baseline Screening**. Please try to remember to carry it with you at all times. If you forget to take the Diary Booklet with you, or are unable to carry it at all times, please make written notes on a pad and transfer them to the booklet as soon as possible. We have asked you to keep **two diaries during this period**:

a) **A 7-day Stool and Symptom Diary.** Each diary day covers a 24 hour period.
   - Please record details of your stools as soon as possible after you have passed them.
   - Please complete the questions about IBS symptoms towards the end of each day, e.g. before you go to bed.

b) **A 7-day Food and Drink Diary.** Please make entries in your booklet throughout the day, rather than from memory at the end of the day. During baseline screening for the study it is **important that you to continue to follow your usual diet**, eating and drinking as you normally would.

This booklet is split into two sections.

Section 1 contains the blank diaries for you to complete. We have organised the diaries in a day-by-day format to make it easier for you to record the right information on the right days. Each diary day covers a 24 hour period.

Section 2 provides additional information on filling out your food diary and information on foods and medications that need to be avoided during the study

Your next appointment

Appointment	Location	Date	Time
Study Visit 1			

How to contact us

If you have any queries please contact

Landline: 020 7848 4447
Mobile: 07794690094
Email: heidi.staudacher@kcl.ac.uk

Day 7 of 7

Day of week.....

Date.....

Every time you open your bowels today please complete the relevant box(es) below. Please refer to the Bristol Stool Chart on the back page.

If you did not have a bowel action today please tick here: ☐

BOWEL MOVEMENT 1

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.* **Type** ☐

BOWEL MOVEMENT 2

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.* **Type** ☐

BOWEL MOVEMENT 3

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.* **Type** ☐

BOWEL MOVEMENT 4

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.* **Type** ☐

BOWEL MOVEMENT 5

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.* **Type** ☐

BOWEL MOVEMENT 6

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.* **Type** ☐

STOOL AND SYMPTOM DIARY

At the end of each day, please rate your symptoms by placing a tick in the box that best describes them. If you do not have this symptom, tick 'absent'

	Absent I didn't have this symptom	**Mild** I had it but it didn't bother me much	**Moderate** It bothered me quite a bit	**Severe** It bothered me a great deal
Abdominal discomfort or pain (discomfort/ pain in your abdomen)	Absent	Mild	Moderate	Severe
Heartburn (burning / discomfort behind your breastbone)	Absent	Mild	Moderate	Severe
Acid reflux / acid regurgitation (taste of sour fluid in your mouth)	Absent	Mild	Moderate	Severe
Nausea (feeling sick, but without vomiting)	Absent	Mild	Moderate	Severe
Abdominal gurgling / rumbling (vibration/ noise in your stomach or belly)	Absent	Mild	Moderate	Severe
Abdominal bloating / distension (swelling in your stomach or belly)	Absent	Mild	Moderate	Severe
Belching / burping (bringing up gas through your mouth)	Absent	Mild	Moderate	Severe
Flatulence / passing wind (release of gas from your bottom)	Absent	Mild	Moderate	Severe
Constipation	Absent	Mild	Moderate	Severe
Diarrhoea	Absent	Mild	Moderate	Severe
Loose stools (mushy or watery stools)	Absent	Mild	Moderate	Severe
Hard stools(lumpy or dry stools)	Absent	Mild	Moderate	Severe
Urgency (urgent need to open your bowels)	Absent	Mild	Moderate	Severe
Incomplete evacuation (feeling of inability to pass all stool)	Absent	Mild	Moderate	Severe
Tiredness	Absent	Mild	Moderate	Severe
Overall symptoms	Absent	Mild	Moderate	Severe

FOOD AND DRINK DIARY **Day of the week** **Date**

Time	Food/drink description and preparation	Brand name	Portion size or quantity eaten
6 am to 9am			
9 am to 12 noon			

Time	Food/drink description and preparation	Brand name	Portion size or quantity eaten
12 noon to 2pm			
2pm to 5pm			

Time	Food/drink description and preparation	Brand name	Portion size or quantity eaten
5pm to 8pm			
8pm to 10pm			
10pm to 6am			

Did you take any **vitamins, minerals or other food supplements** today? Yes ☐ No ☐
If yes, **please describe the supplements you took below**

Brand	Name (in full) including strength	Number of pills, capsules, teaspoons

Please record details of any recipes or ingredients of made up dishes or take-away dishes (if not already described).

NAME OF DISH:		**SERVES:**	
Ingredients	**Amount**	**Ingredients**	**Amount**

Did you have adequate relief of your IBS symptoms over the last 7 days?

Yes No

SECTION TWO
Instructions and guidance

Food and Drink Diary example

Day of the week *Thurs* **Date** *31 March*

Time	Food/drink description and preparation	Brand name	Portion size or quantity eaten
6 am to 9am			
6.30 am *7.30 am*	*Filter coffee, decaffeinated* *Milk (fresh, semi-skimmed).* *Sugar (white)* *Filter coffee with milk and sugar* *Cornflakes* *Milk (fresh, semi-skimmed)* *Toast, granary medium sliced* *Light spread* *Marmalade*	*Douwe Egberts* *Silverspoon* *As above* *Tesco's own* *Hovis* *Flora* *Hartleys*	*Mug* *A little* *1 level tsp* *As above* *Picture 1b* *Drowned* *1 slice* *Med spread* *1 heaped tsp*
9 am to 12 noon			
10.15 am *11 am*	*Instant coffee, not decaffeinated* *Milk (fresh, whole) Sugar brown* *Digestive biscuit – chocolate coated on one side*	*Kenco* *McVities*	*Mug* *A little, 1 level tsp* *2*

Pictures for food portion guidance

Use the pictures to help you indicate the size of the portion you have eaten. Write on the food record the picture number and size A, B or C nearest to your own helping.

Remember that the pictures are much smaller than life size. The actual size of the dinner plate is 10 inches (25cm), the side plate, 7 inches (18cm), and the bowl, 6.3 inches (16cm).

The tables in your food diary guidance booklet also give examples of foods that you might eat and how much information is required about them.

Breakfast cereals

Rice

Spaghetti

Chips

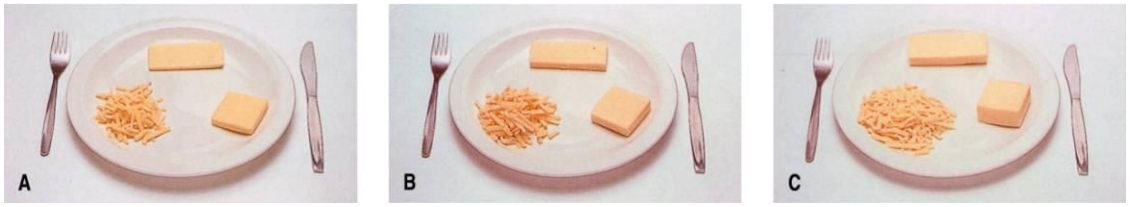
Broccoli or cauliflower

Stew or curry

Battered fish

Quiche or pie

Cheese



Note: in each picture the slice, cube and pile of grated cheese represent the same weight of cheese. If you record the amount of cheese you've eaten as e.g. 'Picture 9a', your record will refer to just one of the three (slice, cube, grated) on the plate in picture 9a.

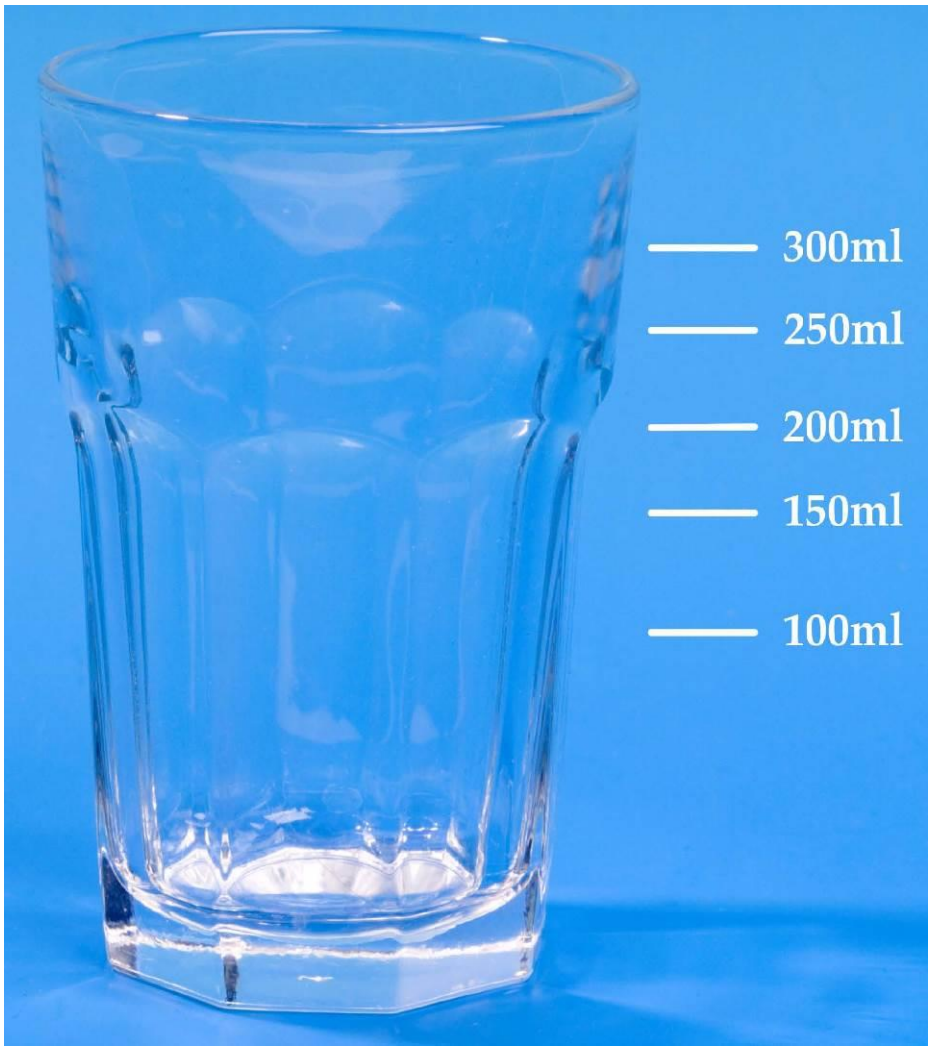
Sponge cake

Typical quantities of drinks in various containers measured in millilitres (ml)

	Small glass	Average glass	Large glass	Vending cup	Cup	Mug
Soft drinks	150	200	300			
Wine	125	175	250			
Hot drinks				170	190	260

Glasses come in different shapes and sizes. On the next page is a life-size glass showing approximate volumes. You can use this picture as a guide for estimating how much volume of drink the glass holds that you are drinking from.

Life size glass



Life size spoons

Teaspoon

(5 ml)

Dessert spoon

(10 ml)

Tablespoon

(15 ml)

Eating and drinking during baseline screening

Before and during the study it is also important that you avoid foods, drinks and supplements that contain probiotics or prebiotics. You can usually tell from the label if there's a prebiotic or probiotic in a food or supplement. These labels will usually contain the following words:

- Prebiotic
- Probiotic
- Bio
- Live culture
- Lactic cultures
- Yogurt cultures
- Lactobacillus
- Acidophilus
- L. acidophilus
- Fructooligosaccharide
- Oligofructose
- Inulin

Yogurt
If you eat yogurt please check the label for the words listed above and select an alternative from the products listed below.

Yogurts to avoid	**Suitable alternatives**
Alpro (soya yogurt)	Müller corners (except Breakfast variety)
Activia	Müller Light
Benecol	Müller Amoré
Bon Maman	Nom
Muller Vitality	Yoplait Yop Drinking Yoghurt
Onken	Yoplait Perle de Lait
Rachel's	Frozen yoghurt e.g. Sainsbury's or Yeo Valley
Shape	Fromage frais (e.g. petit filous) is usually not probiotic and can be used instead of yogurt
Ski	
Total	
Yeo Valley	
Weight Watchers	

.

Other foods, drinks and supplements

Avoid the list of foods below as they contain probiotics or prebiotics. It is not comprehensive – other products may contain prebiotics and/or probiotics.

- Alpen Light Cereal Bars
- ASDA Cholesterol Lowering Yoghurt Drinks
- ASDA Inner Defence Yoghurt Drinks
- ASDA Vitality Cereal Bars
- Bassetts Soft & Chewy Active Health Multivitamins With Minerals
- Benecol Drinking Yoghurt
- Bimuno products
- Boots Probiotic Multivitamins
- Boots Biobalance Support
- Danone Actimel Yoghurt Drinks
- Flora Pro Activ Cholesterol Lowering Health Drinks
- Healthspan Probiotic Capsules
- Healthspan Regulease
- Healthspan Super20 Probiotic
- Holland And Barrett Acidophilus Tablets
- Kellogg's All Bran Breakfast Biscuits
- Kellogg's Fibre Plus Cereal Bars
- Moma Oat Breakfast Products
- Muller Vitality Yoghurt Drinks
- Multibionta Probiotic Multivitamins
- Probio 7 Pre Or Probiotics
- Quaker Morning Bars
- Rice Krispies Multigrain
- Sainsbury's Fruit Of The Forest Yoghurt Slices
- Sainsbury's Probiotic Be Good To Yourself Yoghurt Drinks
- Tesco Fruit And Fibre Cereal Bars
- Tesco Light Choices Cereal Bars
- Tesco Probiotic Yoghurt Drinks
- VSL #3
- Weetabix Oaty Bars
- Weetabix Oatibix Sultana And Apple Bitesize Cereal
- Yakult

Medications and therapies during the study

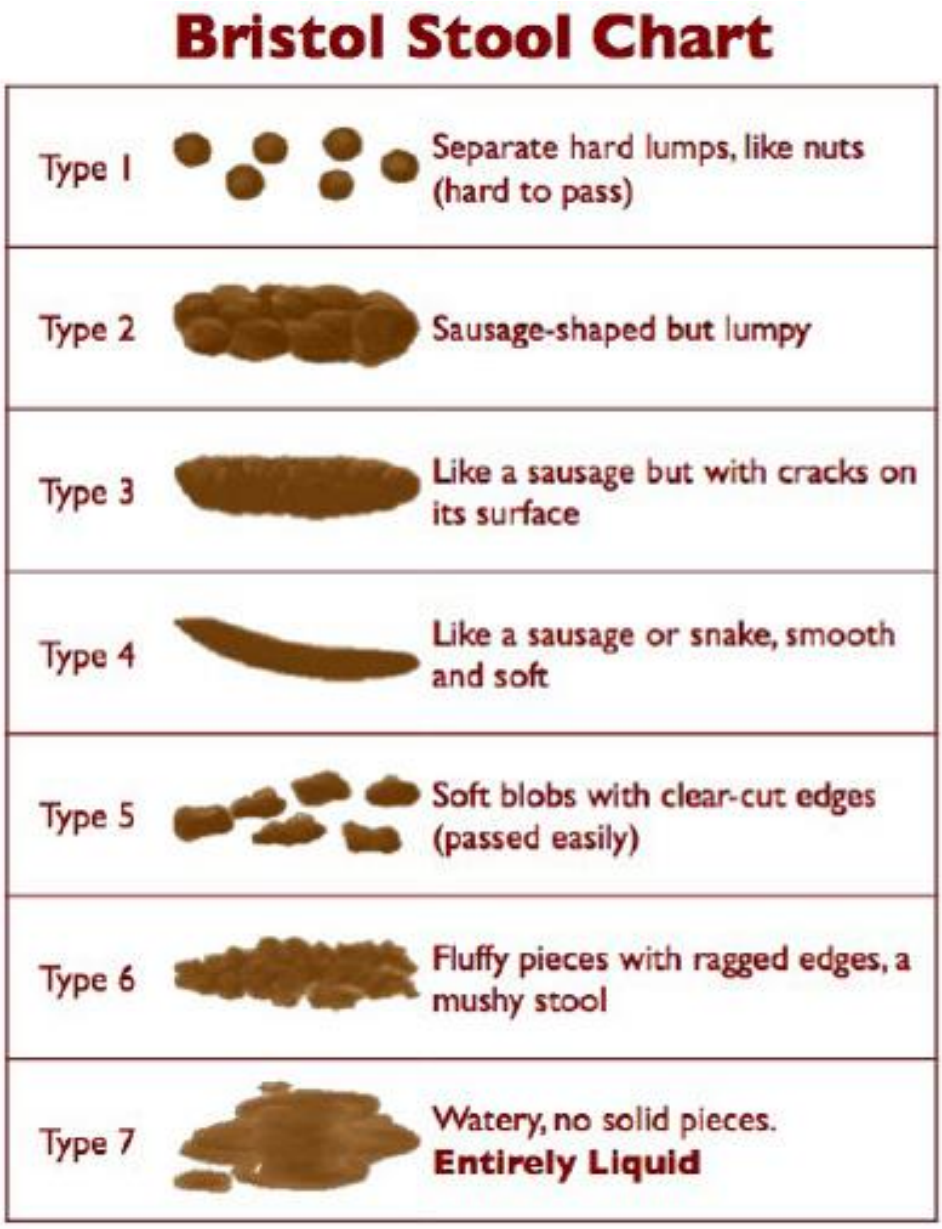
Please tell one of the researchers as soon as possible if you begin taking any of the following medications:

- Antibiotics
- Prebiotics or probiotics
- Bowel preparation for an investigative procedure on your bowel

If there is a change to the dose or type of your IBS medication
If you start taking a new medicine and are unsure whether it is included in this list, please ask one of the researchers.

Bristol Stool Chart

Please refer to this chart each time you complete a Stool and Symptom Diary.



9.6 Instructions on stool collection

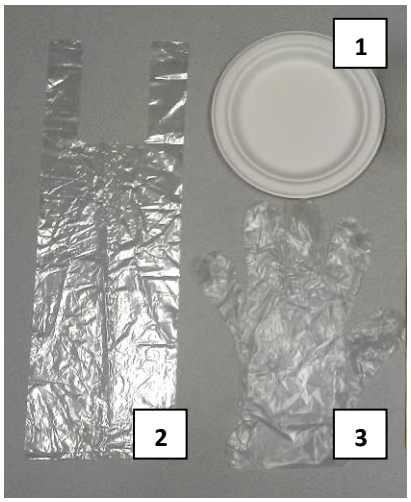
Collecting your stool

During the study we will ask you to collect your stool at Visit 1 and Visit 2. It is best that we collect your stool at the study centre as **we need to start processing it within 1-2 hours of being passed.**

We gather important information from these stool collections, including information about the bacteria in your gut and pH.

Stool collection kit

1. Disposable paper plate
2. Tie-handle clear plastic bags
3. Disposable gloves



How to collect your stool

Most participants will collect stool at the study centre and therefore these instructions will be given to you at your visit.

1. If you need to urinate, please do so before you collect your stool.

   YOU MUST ENSURE THAT YOU DO NOT URINATE AT THE SAME TIME AS COLLECTING YOUR STOOL SAMPLE - this would contaminate the sample.

2. Put on the disposable gloves.

3. Open up a tie-handle clear plastic bag, then flatten the bottom and shape the sides to form a container shape.

4. Sit the opened plastic bag on top of the paper plate. Then put your hands inside the plastic bag, and tuck the base of the bag loosely over the rim of the plate. Please note: the plate should be sitting underneath the bag, not inside it.

5. Starting with the handles, roll down the sides of the bag as shown in the next photo. Roll the bag down to leave about 5 cm (2 inches) of the bag above the plate.

6. Place the plate and bag into the toilet so that the bag remains on the top of the plate. The plate and bag should sit in the toilet bowl and prevent your stool from going into the water.

   Tip: Make sure the plate is stable in the toilet bowl – you may need to press on it a little to make sure it is wedged in place and won't tip when you pass a stool into the plastic bag.

7. Pass your stool. The stool should land inside the plastic bag. REMEMBER - YOU MUST ENSURE THAT YOU DO NOT URINATE AT THE SAME TIME as this would contaminate the sample.

8. Double-bag the sample.

9. Place the sample in the container provided with some ice cubes in the bottom of the container to keep the sample cold.

Pt Id

9.7 IBS-SSS

IBS Severity Scoring System (IBS-SSS)

INSTRUCTIONS

This form is designed to enable us to record and monitor the severity of your IBS. It is to be expected that your symptoms might vary over time, so please try and answer the questions based on how you currently feel (i.e. over the last 10 days).

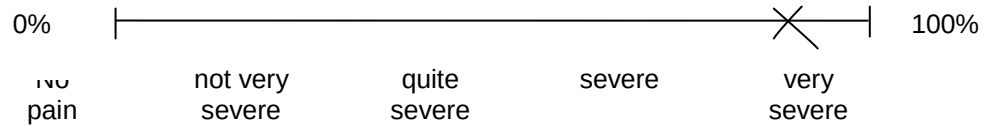
1. For questions where a number of different responses are possible, please circle the response appropriate to you

2. Some questions will require you to write an appropriate response

3. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem.

For example:
How severe was your pain?

Please put a cross (**X**) anywhere on the line between 0-100% in order to indicate as accurately as possible the severity of your symptom. This example shows a severity of appropriately 90%



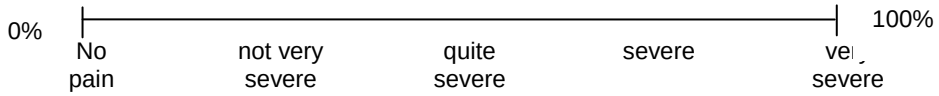
Pt Id

IBS-SSS

1. a) Did you suffer from abdominal (tummy) pain in the last 10 days (please circle)?

Yes No

b) If yes, how severe was/is your abdominal pain



c) Please enter the number of days that you had pain in the last 10 days.

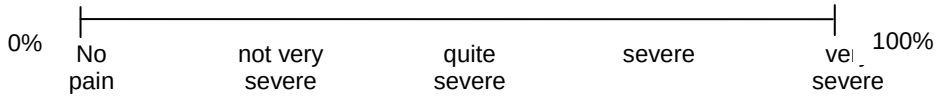
Number of days with pain

2. a) *Did you suffer from abdominal distension (bloated, swollen or tight tummy) in the last 10 days (please circle)?*

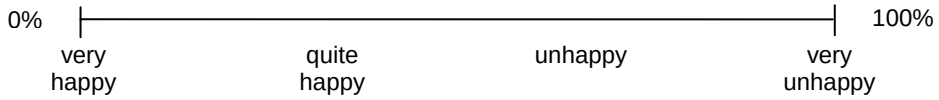
Women please ignore any distension associated with your periods

Yes No

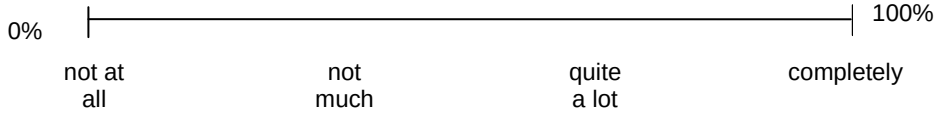
b) If yes, how severe was/is your abdominal distension/tightness



4. How satisfied were you with your bowel habit in the last 10 days?



4. Please indicate with a cross on the line below how much your Irritable Bowel Syndrome was/is affecting or interfering with your life in general over the last 10 days



Pt Id

9.8 SF-36

SF-36 Your Health and Well being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

For each of the following questions, please tick the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very Good	Good	Fair	Poor
☐	☐	☐	☐	☐

2. **Compared to one year ago,** how would you rate your health in general **now**?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
☐	☐	☐	☐	☐

Pt Id ☐

3. The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐	☐	☐
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	☐	☐	☐
Lifting or carrying groceries	☐	☐	☐
Climbing **several** flights of stairs	☐	☐	☐
Climbing **one** flight of stairs	☐	☐	☐
Bending, kneeling or stooping	☐	☐	☐
Walking **more than a** mile	☐	☐	☐
Walking **several blocks**	☐	☐	☐
Walking **one block**	☐	☐	☐
Bathing or dressing yourself	☐	☐	☐

Pt Id ☐

4. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
Cut down the amount of time you spent on work or other activities?	☐	☐
Accomplished less than you would like?	☐	☐
Were limited in the **kind** of work or other activities	☐	☐
Had **difficulty** performing the work or other activities (for example, it took extra effort)	☐	☐

5. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
Cut down on the **amount of time** you spent on work or other activities?	☐	☐
Accomplished less than you would like?	☐	☐
Didn't do work or other activities as **carefully** as usual	☐	☐

Pt Id ☐

6. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐	☐	☐	☐	☐

7. How much **bodily pain** have you had during the **past 4 weeks**?

None	Very mild	Mild	Moderate	Severe	Very severe
☐	☐	☐	☐	☐	☐

8. During the **past 4 weeks,** how much did **pain** interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐	☐	☐	☐	☐

Pt Id ☐

9. These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks . . .**

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
Did you feel full of pep?	☐	☐	☐	☐	☐	☐
Have you been a very nervous person?	☐	☐	☐	☐	☐	☐
Have you felt so down in the dumps that nothing could cheer you up?	☐	☐	☐	☐	☐	☐
Have you felt calm and peaceful?	☐	☐	☐	☐	☐	☐
Did you have a lot of energy?	☐	☐	☐	☐	☐	☐
Have you felt downhearted and blue?	☐	☐	☐	☐	☐	☐
Did you feel worn out?	☐	☐	☐	☐	☐	☐
Have you been a happy person?	☐	☐	☐	☐	☐	☐
Did you feel tired?	☐	☐	☐	☐	☐	☐

Pt Id ☐

10. During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting friends, relatives, etc)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐	☐	☐	☐	☐

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
I seem to get sick a little easier than other people	☐	☐	☐	☐	☐
I am as healthy as anybody I know	☐	☐	☐	☐	☐
I expect my health to get worse	☐	☐	☐	☐	☐
My health is excellent	☐	☐	☐	☐	☐

9.9 IBS-QOL

PLEASE WRITE IN
TODAY'S DATE: _____ _____ _____
DAY MONTH YEAR

PARTICIPANT/PATIENT ID:

PLEASE READ THIS CAREFULLY

ON THE FOLLOWING PAGES YOU WILL FIND STATEMENTS CONCERNING BOWEL PROBLEMS (IRRITABLE BOWEL SYNDROME) AND HOW THEY AFFECT YOU.

FOR EACH STATEMENT, PLEASE CHOOSE THE RESPONSE THAT BEST APPLIES TO YOU AND **CIRCLE** THE NUMBER OF YOUR RESPONSE.

IF YOU ARE UNSURE ABOUT HOW TO RESPOND TO A STATEMENT, PLEASE GIVE THE BEST RESPONSE YOU CAN. **THERE ARE NO RIGHT OR WRONG RESPONSES.**

YOUR RESPONSES WILL BE KEPT STRICTLY CONFIDENTIAL.

IF YOU HAVE ANY QUESTIONS, PLEASE CONTACT:

SITE ADDRESS AND PHONE NUMBER TO BE PLACED HERE

Pt Id

About Your Feelings

Please think about your life over the **past month (last 30 days)** and look at the statements below. Each statement has five possible responses. For each statement, please circle the one response that best describes your feelings.

1. I feel helpless because of my bowel problems. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 EXTREMELY

2. I am embarrassed by the smell caused by my bowel problems. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 EXTREMELY

3. I am bothered by how much time I spend on the toilet. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 A GREAT DEAL

4. I feel vulnerable to other illnesses because of my bowel problems. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 EXTREMELY

5. I feel fat or bloated because of my bowel problems. *(Please circle one number)*

   1 NOT AT ALL
   2 SLIGHTLY
   3 MODERATELY
   4 QUITE A BIT
   5 A GREAT DEAL

6. I feel as though I am losing control of my life because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

7. I feel that my life is less enjoyable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

8. I feel uncomfortable when I talk about my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

9. I feel depressed about my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

10. I feel isolated from other people because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

11. I have to be careful about the amount of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

12. Because of my bowel problems sexual activity is difficult for me. *(Please circle one number)*
(If not applicable, please circle "NOT AT ALL")

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

13. I feel angry that I have bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

14. I feel as though I irritate others because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

15. I worry that my bowel problems will get worse. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

16. I feel irritable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

17. I worry that people think I exaggerate my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

18. I feel that I get less done because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

19. I have to avoid stressful situations because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

20. My bowel problems reduce my sexual desire. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

21. My bowel problems limit what I can wear. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

22. I have to avoid strenuous activity because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

23. I have to be careful about the kind of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

24. Because of my bowel problems I have difficulty being with unfamiliar people. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

25. I feel sluggish because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

26. I feel "unclean" because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

27. Long trips are difficult for me because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

28. I feel frustrated that I cannot eat when I want to because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

29. It is important to be near a toilet because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

30. My life revolves around my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

31. I worry about losing control of my bowels. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

32. I am afraid that I won't be able to have a bowel movement. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

33. My bowel problems are affecting my closest relationships. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

34. I feel that no one understands my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Pt Id

9.10 Acceptability questionnaire

Acceptability Questionnaire

Please circle the answer most applicable to you. While following the research diet:

1. Meal preparation was

-3	-2	-1	0	+1	+2	+3
Much More Difficult	More Difficult	Slightly More Difficult	No Different	Slightly Easier	Easier	Much Easier

2. The time I spent shopping for food was

-3	-2	-1	0	+1	+2	+3
Much Longer	Longer	Slightly Longer	No Different	Slightly Shorter	Shorter	Much Shorter

3. The time I spent preparing and cooking meals and snacks was

-3	-2	-1	0	+1	+2	+3
Much Longer	Longer	Slightly Longer	No Different	Slightly Shorter	Shorter	Much Shorter

4. Finding suitable food choices when eating out was

-3	-2	-1	0	+1	+2	+3
Much More Difficult	More Difficult	Slightly More Difficult	No Different	Slightly Easier	Easier	Much Easier

5. I found the flavour of the meals and snacks to be

-3	-2	-1	0	+1	+2	+3
Much Less Appealing	Less Appealing	Slightly Less Appealing	No Different	Slightly More Appealing	More Appealing	Much More Appealing

5. The money I spent on grocery shopping and eating out compared to my usual diet was

-3	-2	-1	0	+1	+2	+3
Much More Expensive	More Expensive	Slightly More Expensive	No Different	Slightly Cheaper	Cheaper	Much Cheaper

Pt Id

7. Understanding the written information given to me was

-3	-2	-1	0	+1	+2	+3
Very Difficult to Understand	Difficult to Understand	Quite Difficult to Understand	Neutral	Quite Easy to Understand	Easy to Understand	Very Easy to Understand

8. How convenient was this diet compared to your normal diet?

-3	-2	-1	0	+1	+2	+3
Much Less Convenient	Less Convenient	Slightly Less Convenient	No Different	Slightly More Convenient	More Convenient	Much More Convenient

9. How troublesome or difficult was this diet compared to your normal diet?

-3	-2	-1	0	+1	+2	+3
Much More Difficult Than Normal	More Difficult Than Normal	Slightly More Difficult Than Normal	No Different To Normal	Slightly Easier Than Normal	Easier Than Normal	Much Easier Than Normal

10. Did any benefits of being involved in this study outweigh the burden of being on this diet? (Please circle)

Yes **No**

11. Overall did you have to make many changes to your diet? (Please circle)

Yes **No**

12. Was there a food or drink that you missed a lot? (Please circle)

Yes **No**

13. If yes, what food or drink was it? _____

Pt Id

The following questions relate to the probiotic/placebo. Please circle the answer most applicable to you.

1. Regarding taking the sachets daily for 4 weeks, was it

Acceptable/easy Difficult

2. Did you get any side effects from taking the probiotic/placebo sachets?

Yes No

If so, please provide detail:

_____

_____

3. Did you know what a probiotic was prior to this trial?

Yes No

4. Would you classify a probiotic as a

Standard treatment Complementary/ alternative medicine Neither

5. Would you take a probiotic for your IBS symptoms in the future?

Yes No

9.11 Diet record guidance

Food and Drink Guidance Booklet

Instructions for completing your Food and Drink Diary

PLEASE READ THROUGH THIS BOOKLET BEFORE STARTING YOUR FOOD AND DRINK DIARY

This **Food and Drink Diary Guidance Booklet** provides additional information about recording what you eat and drink.

Completing your diary:

1. **Day and date**
   Please write down the day and date at the top of the page each time you start a new day of recording.

2. **Time slots**
   Please note the time of each eating occasion into the space provided. For easy use each day is divided into sections, from the first thing in the morning to late evening and through the night.

3. **What do you eat?**
   Please describe the food you eat in as much detail as possible. Be as specific as you can. Pages 7-14 of this booklet will help with the sort of detail we need, like cooking methods (fried, grilled, baked etc) and any additions (fats, sugar/sweeteners, sauces, pepper etc).

4. **Homemade dishes**
   If you have eaten any homemade dishes e.g. chicken casserole, please record the name of the recipe, ingredients with amounts (including water or other fluids) for the whole recipe, the number of people the recipe serves, and the cooking method. Write this down in the recipe section at the end of the record day. Record how much of the whole recipe you have eaten in the portion size column.

5. **Takeaways and eating out**
   If you have eaten takeaways or made up dishes not prepared at home such as at a restaurant or a friend's house, please record as much detail about the ingredients as you can e.g. vegetable curry containing chickpeas, aubergine, onion and tomato.

6. **Brand name**
   Please note the brand name (if known). Most packed foods will list a brand name, e.g. Bird's eye, Hovis, or Supermarket own brands.

7. **Portion sizes**
   Examples for how to describe the quantity or portion size you had of a particular food or drink are shown in this booklet.

   For **foods**, quantity can be described using:

- Household measures, e.g. one teaspoon (tsp) of sugar, two thick slices of bread, 4 tablespoons (tbsp) of peas, ½ cup of gravy. Be careful when describing amounts in spoons that you are referring to the correct spoon size. Compare the spoons you use with the life size pictures in your participant diary.
- Weights from labels, e.g. 4oz steak, 420g tin of baked beans, 125g pot of yogurt.
- Number of items, e.g. 4 fish fingers, 2 pieces of chicken nuggets, 1 regular size jam filled doughnut .
- Picture examples for specific foods in your diary booklet.

For **drinks**, quantity can be described using:

- The size of glass, cup etc (e.g. large glass) or the volume (e.g. 300ml). Examples of typical drinks containers are in your diary booklet.
- Volumes from labels (e.g. 330ml can of fizzy drink).

We would like to know the amount that was actually consumed which means **taking leftovers into account**. You can do this in two ways:

- Record what was served and make notes of what was not eaten e.g. 3 tbs of peas, only 2 tbs eaten; 1 large sausage roll, ate only ½ .
- Only record the amount actually eaten i.e. 2 tbs of peas, ½ a large sausage roll.

8. **Supplements**
   At the end of each recording day there is a section for providing information about any supplements you took. Brand name, full name of supplement, strength and the amount taken should be recorded.

9. **When to fill in the diary**
   Please record your eating and drinking as you go, not from memory at the end of the day. Use written notes on a pad if you forget to take your diary with you. Each diary day covers a 24 hour period, so please include any food or drinks that you may have had during the night. Remember to include foods and drinks between meals (snacks) including water.

10. **Completed diary examples**
    On pages 3-6 of this booklet you can see an example day that have been filled in. This shows you how we would like you to record your food and drink, for example a ready meal and a homemade dish.

With thanks to the Medical Research Council for Human Nutrition Research for permission to use this Food and Drink Diary design.

Food and Drink Diary example

Day of the week *Thurs* **Date** *31 March*

Time	Food/drink description and preparation	Brand name	Portion size or quantity eaten
6 am to 11am			
6.30 am *7.30 am* 	*Filter coffee, decaffeinated* *Milk (fresh, semi-skimmed).* *Sugar (white)* *Filter coffee with milk and sugar* *Cornflakes* *Milk (fresh, semi-skimmed)* *Toast, granary medium sliced* *Light spread* *Marmalade*	*Douwe Egberts* *Silverspoon* *As above* *Tesco's own* *Hovis* *Flora* *Hartleys*	*Mug* *A little* *1 level tsp* *As above* *Picture 1b* *Drowned* *1 slice* *Med spread* *1 heaped tsp*
10.15 am *11 am* 	*Instant coffee, not decaffeinated* *Milk (fresh, whole) Sugar brown* *Digestive biscuit – chocolate coated on one side*	*Kenco* *McVities*	*Mug* *A little* *1 level tsp*

Did you take any **vitamins, minerals or other food supplements** today? Yes ☐ No ☐

Brand	Name (in full) including strength	Number of pills, capsules, teaspoons
Holland & Barrett	*Evening Primrose Oil – 1000 mg*	*1 capsule*
Holland & Barrett	*Vitamin E – 400 IU*	*1 capsule*

Please record details of any recipes or ingredients of made up dishes or take-away dishes (if not already described).

NAME OF DISH: *Fairy cakes* **SERVES:** *makes 20 cakes*

Ingredients	Amount	Ingredients	Amount
Tate & Lyle caster sugar	*175g*	*Silver Spoon icing sugar*	*140g*
Anchor butter, unsalted	*175g*	*Yellow food colouring*	*3 drops*
Eggs	*3*	*Water*	*2 tablespoons*
Homepride self-raising flour	*175g*		
Baking powder	*1 teaspoon*		

Brief description of cooking method

Mix together and bake for 15 min.

Mix icing sugar with water and add colouring. Approx. 1 teaspoon of icing on each cake

Examples and advice on food descriptions

This table explains the detail we'd like you to provide in your Food and Drink Diary for the different foods and drinks listed.

Food/drink	Description and preparation	Portion size or quantity
Bacon	Back, middle, streaky; smoked or un-smoked; fat eaten; dry-fried or fried in oil/fat (type used) or grilled rashers	Number of rashers
Baked beans	Standard, reduced salt or reduced sugar	Tablespoons, weight of beans marked on tin label (e.g. 420g)
Beefburger (hamburger)	Home-made (ingredients), from a packet (brand name) or take-away; fried (type of oil/fat), microwaved or grilled; economy; with or without bread roll	Number, large or small, ounces or in grams if info on package
Beer	What sort e.g. stout, bitter, lager; draught, canned, bottled; low-alcohol or home-made	Number of pints or half pints, size of can or bottle
Biscuits	What sort and brand e.g. cheese, wafer, crispbread, sweet, chocolate, shortbread, home-made	Number, size (standard or mini variety)
Bread (see also sandwiches)	Wholemeal, granary, white or brown; currant, fruit, malt; large or small loaf; sliced or unsliced loaf; give brand	Number of slices; thick, medium or thin slices

Food/drink	Description and preparation	Portion size or quantity
Bread rolls	Wholemeal, white or brown; alone or with filling; crusty or soft	Size, number of rolls
Breakfast cereal (see also porridge)	What sort and brand e.g. Kellogg's cornflakes; any added fruit and/or nuts; Muesli – added sugar and/or fruit	Tablespoons or picture 1
Bun	Iced, currant or plain, homemade or bought (brand name)	Large or small, number
Butter, margarine & fat spreads	Give full product name	Thick/average/thin spread; spoons
Cake	Individual or piece of large; type and brand; fruit (rich), sponge, fresh cream, buttercream, iced; type of filling	Number, slices, packet weight, see picture 10 for sponge cake
Cheese	Name, brand and type e.g. cheddar, cream, cottage, soft; low fat	picture 9, or number of slices, thick or thin cut, number of spoons
Chips	Fresh, frozen, oven, microwave, take-away (where from); thick/straight/crinkle/fine cut; type of oil/fat used for cooking give brand name	picture 4, as A, B, or C or 2 x B, etc
Chocolate(s)	What sort e.g. plain, milk, white, fancy, diabetic; type of filling; give brand name	Number, weight/size of bar

Food/drink	Description and preparation	Portion size or quantity
Coffee	With milk (see section on milk); half milk/half water; all milk; ground/filter, instant; decaffeinated; give brand name	Cups or mugs
Cream	Single, whipped, double or clotted; dairy or non-dairy; low-fat; fresh, UHT/Longlife; imitation cream e.g. Elmlea	Tablespoons
Crisps	What sort e.g. potato, corn, wheat, maize, vegetable etc; give brand; flavour; low-fat or low-salt; premium variety e.g. Kettle chips, Walker's Sensations	Packet weight
Custard	Pouring custard or egg custard; made with powder and milk/sugar, instant, ready to serve (tinned or carton); low fat, sugar free, brand	Tablespoons
Doughnut	Plain, jam, cream or iced; round or ring, where bought/brand name	Number, size e.g. mini, large
Egg	Boiled, fried (type of oil/fat), scrambled (type of fat used, with or without added milk), poached, omelette (with or without filling, type of oil/fat used), etc	Number of eggs, large, medium or small eggs

Food/drink	Description and preparation	Portion size or quantity
Fish (including canned)	What sort and brand e.g. cod, tuna; fried (type of oil/fat), grilled, poached (water or milk) or steamed; with batter or breadcrumbs; canned in oil, brine or tomato sauce	Size of can or spoons (for canned fish) or picture 7 for battered fish
Fish cakes & fish fingers	Type of fish; plain or battered or in breadcrumbs; fried, grilled, baked or microwaved; economy	Size, number, packet weight
Fruit -fresh	What sort; eaten with or without skin	Size, number
Fruit -stewed/canned	What sort; sweetened or unsweetened; in fruit juice or syrup; juice or syrup eaten	Tablespoons Size of can or weight on can
Fruit – juice (pure)	What sort and brand e.g. apple, orange; sweetened or unsweetened; pasteurised or UHT/Longlife; freshly squeezed; added vitamins/minerals, omega 3?	Glass (size or volume) or carton size
Ice cream	Flavour; dairy or non-dairy; brand name; luxury/premium; added nuts, fruit	Number of tablespoons/ scoops
Jam, honey	What sort; low-sugar/diabetic; shop bought/brand or homemade	Teaspoons, heaped or level, or thin or thick spread
Marmalade	Type and brand; low-sugar; thick cut; shop bought/brand or homemade	Teaspoons, heaped or level, or thin or thick spread

Food/drink	Description and preparation	Portion size or quantity
Meat (see also bacon, burgers & sausages)	What sort; cut of meat e.g. chop, breast, minced; lean or fatty; fat removed or eaten; skin removed or eaten; how cooked; with or without gravy	Large/small/medium, tablespoons, or picture 6 for stew portion
Milk	Brand and type (whole, semi-skimmed, skimmed); fresh, sterilized, UHT, dried; soya milk (sweetened/unsweetened), goats' milk, rice milk; flavoured; fortified with added vitamins and/or minerals	Pints, glass (size or volume) or cup. For milk on cereal: *damp/normal/drowned.* For milk in tea/coffee: *a little/some/a lot*
Nuts	What sort and brand; dry roasted, ordinary salted, honey roasted; unsalted	Packet weight, handful
Pie (sweet or savoury)	What sort and brand; individual or helping; one pastry crust or two; type of pastry	Individual or slice, or picture 8
Pizza	Thin base or deep pan or French bread; topping; brand name and type	Individual, slice, fraction of large pizza e.g. ¼
Porridge	Brand name; made with oats or cornmeal or instant oat cereal; made with milk and/or water; with sugar or honey; with milk or cream	Bowls

Food/drink	Description and preparation	Portion size or quantity
Potatoes (see also chips)	Old or new; baked, boiled, roast (type of oil/fat); skin eaten; mashed (with butter/spread and with or without milk); fried/chips (type of oil/fat); instant; any additions e.g. butter	Mash – tablespoons, number of half or whole potatoes, small or large potatoes, or picture 4 for chips portion
Pudding	What sort; e.g. steamed sponge; with fruit; mousse; instant desserts; milk puddings	Tablespoons, picture 10 for slice of sponge
Rice	What sort; e.g. basmati, easy cook, long or short grain; white or brown; boiled or fried (type of oil/fat); brand name	Tablespoons or picture 2
Salad	Ingredients; if with dressing what sort (oil and vinegar, mayonnaise); brand name of dressing	Amount of each component; e.g. number of tomatoes, slices of cucumber, leaves; tablespoons of dressing
Sandwiches and rolls	Type of bread/roll (see Bread & Rolls); butter or margarine; type of filling; including salad, mayonnaise, pickle etc. If shop-bought, where from?	Number of rolls or slices of bread; amount of butter/margarine (on both slices?); amount of filling
Sauce – cold (including mayonnaise)	Tomato ketchup, brown sauce, soy sauce, salad cream, mayonnaise; low fat; brand name	Teaspoons, tablespoons

Food/drink	Description and preparation	Portion size or quantity
Sausages	What sort; e.g. beef, pork; fried (type of oil/fat) or grilled; low fat; economy; brand name	Large or small, number
Sausage rolls	Type of pastry; brand name	Number, size e.g. jumbo, standard, mini
Scone	Fruit, sweet, plain, cheese; type of flour; bought/brand or homemade	Number, small, medium or large
Savoury snacks -in packet	What sort: e.g. Cheddars, cheese straws, Twiglets, Pretzels; give brand name	Size (standard or mini variety), packet weight, number
Soft drinks – squash/ concentrate/cordial	Give brand name & flavour; no added sugar/low calorie/sugar free; “high” juice; fortified with added vitamins and/or minerals	Glass (size or volume)
Soft drinks – carbonated/fizzy	Give brand & flavour; diet/low-calorie; canned or bottled; cola – caffeine free	Glass, can or bottle (size or volume)
Soft drinks – ready to drink	Give brand & flavour; no added sugar/low calorie/sugar free; does it contain real fruit juice, if so, how much?; fortified with added vitamins and/or minerals	Glass, carton or bottle (size or volume)

Food/drink	Description and preparation	Portion size or quantity
Soup	What sort; give brand name; cream or clear; canned, packet, instant or vending machine, home-made	Tablespoons, bowl or mug
Spaghetti, other pasta	What sort; fresh/chilled or dried; white, wholemeal; canned in sauce; type of filling if ravioli, cannelloni etc	Tablespoons (or how much dry pasta used per portion in grams/packet size) or picture 3
Spirits	What sort: e.g. whisky, gin, vodka, rum	Measures as in pub
Sugar	Added to cereals, tea, coffee, fruit, etc; what sort; e.g. white, brown, demerara	Heaped or level teaspoons
Sweets	What sort: e.g. toffees, boiled sweets, diabetic; give brand name	Number, packet weight
Tea	With/without milk (see section on milk); decaffeinated, herb	Mugs or cups
Vegetables (not including potatoes)	What sort; how cooked or raw; additions e.g. butter, other fat or sauce	Tablespoons, number of florets or sprouts, weight from tins or packet as guidance
Water	Tap, filtered, bottled: give brand name	Glass or bottle (size or volume)
Wine, sherry, port	White, red; sweet, dry; low-alcohol; give brand name	Glass (size or volume)

Food/drink	Description and preparation	Portion size or quantity
Yoghurt, fromage frais	What sort: e.g. natural/plain or flavoured; creamy, Greek, low-fat, very low fat/diet, soya; with fruit pieces or just fruit flavoured; twinpot with separate cereal/crumble; fortified with added vitamins and/or minerals; brand name	Pot size or tablespoons
Home-made dishes	Please say what the dish is called (record recipe or details of dish if you can in the section provided) and how many persons it serves	Tablespoons – heaped or level, number, size
Ready-made meals	Please give brand name and full description of product; did it contain any accompaniments e.g. rice, vegetables, sauces; was it chilled or frozen; microwaved, oven cooked, boil-in-the-bag; was it low fat, healthy eating range. Enclose label and ingredients list if possible in your plastic bag	Packet weight, if not whole packet describe portion consumed
Take-away food or food eaten out	Please say what the dish is called and give main ingredients if you can. Give name of a chain restaurant e.g. McDonalds	Tablespoons, portion size e.g. small/medium/large

9.12 Demographic data and nutrient intake for the sham diet pilot study

Demographic data and nutrient intake for the sham diet pilot study in healthy individuals (n=7) to be implemented in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation in patients with IBS

Demographic data

Variable	Mean (SD)
Age yrs	33 (7)
Females n (%)	6 (86)
Weight kg	71 (19)
BMI kg/m^2	26 (7)

Values are mean (SD)

Mean (SD) for total energy, nutrient and FODMAP intake of habitual and sham diet

Nutrient	Habitual diet	Sham diet	p[#]
Energy (kcal/d)	2108 (619)	2041 (779)	0.735
Carbohydrate (g/d)	244 (47)	234 (99)	0.499
Starch (g/d)	135 (41)	118 (32)	0.398
Sugars (g/d)	89 (37)	99 (37)	0.499
Protein (g/d)	78 (26)	86 (21)	0.735
Fat (g/d)	80 (24)	80 (42)	0.866
NSP (g/d)	13.4 (3.6)	17.3 (4.7)	**0.043**
FODMAPs			
Total FODMAPs (g/d)	15.8 (5.1)	22.7 (8.5)	0.091
Fructans (g/d)	2.3 (0.9)	3.7 (2.4)	0.176
GOS (g/d)	0.7 (0.4)	0.9 (0.6)	0.310
Sorbitol (g/d)	0.4 (0.6)	1.3 (0.6)	0.091
Mannitol (g/d)	0.3 (0.4)	0.5 (0.3)	0.345
Excess fructose (g/d)	1.4 (0.9)	1.4 (0.7)	1.000
Lactose (g/d)	9.3 (5.1)	11.8 (7.7)	0.237

Values are mean (SD). [#]Wilcoxon signed rank test

9.13 IBS-SSS total and subscores at baseline and follow up

IBS-SSS scores for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Sham diet (n=53)		Low FODMAP diet (n=51)		Placebo (n=51)		Probiotic (n=53)	
	Baseline	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**
IBS-SSS total (pts)	268 (72)	224 (89)	291 (76)	173 (95)	284 (75)	207 (98)	275 (75)	192 (93)
Pain severity	51 (25)	40 (23)	54 (21)	33 (24)	55 (25)	38 (24)	49 (21)	35 (24)
Days of pain (days)	54 (31)	44 (29)	59 (30)	30 (27)	58 (30)	39 (28)	55 (31)	35 (30)
Distension severity	46 (24)	40 (24)	51 (25)	29 (25)	48 (22)	34 (24)	48 (27)	35 (26)
Satisfaction with bowels	59 (18)	53 (17)	65 (19)	42 (23)	62 (18)	49 (22)	62 (19)	46 (20)
Affecting life	58 (17)	47 (21)	62 (19)	40 (20)	61 (20)	46 (21)	59 (17)	41 (20)

Units are (mm) unless stated. Values are mean (SD)

9.14 IBS-SSS scores for intervention combinations

IBS-SSS scores for the intervention combinations at follow up for the intention to treat population for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of the low FODMAP diet and probiotic supplementation

	Sham diet + placebo (n=27)	Sham diet+ probiotic (n=26)	Low FODMAP diet + placebo (n=24)	Low FODMAP diet + probiotic (n=27)	p[#]
IBS-SSS total (pts)	233 (93)	215 (86)	177 (97)	170 (95)	**0.044***
Pain severity	42 (25)	38 (22)	33 (23)	33 (26)	0.426
Days of pain (days)	44 (28)	43 (31)	33 (26)	27 (27)	0.087
Distension severity	41 (24)	39 (25)	26 (23)	31 (28)	0.103
Satisfaction with bowels	54 (17)	53 (16)	45 (25)	39 (21)	**0.024***
Affecting life	51 (21)	42 (20)	40 (20)	41 (21)	0.141
Change in IBS-SSS (pts)	-39 (74)	-49 (70)	-121 (101)	-114 (72)	**<0.001****

Units are (mm) unless stated. Values are mean (SD). No differences at baseline.
One-way ANOVA with Tukey's post hoc testing or Welch test with Games-Howell post hoc testing where homogeneity of variances was not met
*no differences post hoc

**Sham + placebo vs low FODMAP + placebo p=0.011
Sham + placebo vs low FODMAP + probiotic p=0.002
Sham + probiotic vs low FODMAP + placebo p =0.029
Sham + probiotic vs low FODMAP + probiotic p = 0.009

Outcomes for those achieving the minimal clinically important difference in IBS-SSS score for intervention combinations at follow up for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of the low FODMAP diet and probiotic supplementation

	Sham diet + placebo (n=27)	Sham diet + probiotic (n=26)	Low FODMAP diet + placebo (n=24)	Low FODMAP diet + probiotic (n=27)	p[#]
Achieving MCID n (%)	10 (37)	12 (46)	17 (71)	20 (74)	**0.013**

Chi-squared test

9.15 GSRS incidence and severity scores at baseline and follow up

GSRS incidence scores for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Sham diet n=53		Low FODMAP diet n=51		Placebo n=51		Probiotic n=53	
	Baseline	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**
Abdominal pain	2.9 (2.2)	2.1 (2.0)	3.0 (2.4)	1.5 (1.9)	3.1 (2.3)	1.9 (2.1)	2.8 (2.3)	1.7 (2.0)
Heartburn	07 (1.5)	0.2 (0.6)	0.8 (1.8)	0.4 (1.1)	0.8 (1.1)	0.2 (0.6)	0.7 (1.6)	0.4 (1.1)
Acid reflux	0.5 (1.4)	0.3 (0.7)	0.6 (1.3)	0.4 (0.9)	0.6 (1.5)	0.3 (0.6)	0.5 (1.2)	0.4 (0.9)
Nausea	0.6 (1.2)	0.6 (1.4)	0.6 (1.3)	0.3 (0.9)	0.7(1.5)	0.6 (1.6)	0.5 (1.0)	0.3 (0.6)
Borborygmi	2.2 (2.0)	1.9 (2.2)	2.1 (2.4)	1.0 (1.9)	2.3 (2.3)	1.6 (2.2)	2.3 (2.3)	1.4 (2.1)
Bloating	2.6 (2.5)	2.2 (2.3)	3.1 (2.6)	1.5 (2.0)	2.7 (2.4)	1.9 (2.2)	2.1 (2.1)	1.8 (2.2)
Belching	1.1 (1.7)	1.9 (1.8)	1.1 (1.7)	0.6 (1.4)	1.1 (1.8)	0.9 (1.8)	1.4 (2.0)	0.8 (1.6)
Flatulence	3.2 (2.6)	2.7 (2.4)	1.4 (2.1)	1.5 (2.0)	3.3 (2.6)	2.6 (2.4)	2.9 (2.5)	1.7 (2.0)
Constipation	0.8 (1.7)	0.3 (0.7)	0.4 (1.0)	0.4 (1.2)	0.7 (1.6)	0.3 (1.0)	0.5 (1.2)	0.4 (0.9)
Diarrhoea	0.6 (1.3)	0.5 (1.2)	0.8 (1.5)	0.5 (1.5)	0.9 (1.5)	0.7 (1.5)	0.5 (1.3)	0.4 (1.1)
Loose stool	1.9 (2.0)	1.3 (1.8)	1.9 (2.0)	1.1 (1.9)	2.1 (2.3)	1.4 (2.1)	1.7 (1.7)	1.1 (1.6)
Hard stool	0.4 (1.0)	0.2 (0.6)	0.3 (0.8)	0.3 (0.7)	0.3 (0.8)	0.1 (0.4)	0.4 (0.9)	0.4 (0.8)
Urgency	1.7 (1.8)	1.6 (1.8)	2.7 (2.2)	1.2 (1.9)	2.5 (2.1)	1.5(2.0)	1.9 (2.0)	1.4 (1.8)
Incomplete evacuation	1.7 (2.1)	1.7 (2.2)	1.9 (2.3)	0.7 (1.4)	1.8 (2.2)	1.3 (2.1)	1.8 (2.2)	1.2 (1.8)
Tiredness	2.9 (2.6)	2.8 (2.4)	2.7 (2.8)	2.0 (2.5)	3.0 (2.7)	2.6 (2.6)	2.7 (2.7)	2.2 (2.3)
Overall	2.8 (2.4)	2.3 (2.5)	3.1 (2.4)	1.6 (1.9)	2.8 (2.3)	2.3 (2.5)	3.1 (2.5)	1.7 (2.0)

Values are mean (SD) of the number of days on which the symptoms were present in seven days

GSRS severity scores for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Sham diet n=53		Low FODMAP diet n=51		Placebo n=51		Probiotic n=53	
	Baseline	Follow up	Baseline	Follow up	Baseline	Follow up	Baseline	Follow up
Abdominal pain	1.3 (0.6)	1.1 (0.6)	1.4 (0.7)	0.9 (0.7)	1.4 (0.6)	1.0 (0.7)	1.3 (0.6)	0.9 (0.6)
Heartburn	0.3 (0.5)	0.2 (0.3)	0.4 (0.6)	0.2 (0.5)	0.4 (0.6)	0.2 (0.3)	0.3 (0.6)	0.2 (0.5)
Acid reflux	0.3 (0.5)	0.2 (0.5)	0.3 (0.5)	0.2 (0.4)	0.3 (0.6)	0.2 (0.3)	0.3 (0.5)	0.2 (0.4)
Nausea	0.4 (0.5)	0.3 (0.5)	0.4 (0.5)	0.3 (0.4)	0.4 (0.6)	0.3 (0.6)	0.4 (0.5)	0.2 (0.3)
Borborygmi	1.1 (0.6)	1.0 (0.7)	1.1 (0.7)	0.7 (0.6)	1.2 (0.7)	0.9 (0.7)	1.1 (0.6)	0.8 (0.7)
Bloating	1.2 (0.7)	1.1 (0.7)	1.3 (0.9)	0.8 (0.7)	1.3 (0.8)	1.0 (0.7)	1.3 (0.8)	1.0 (0.7)
Belching	0.6 (0.7)	0.6 (0.7)	0.8 (0.7)	0.5 (0.6)	0.7 (0.7)	0.6 (0.7)	0.7 (0.7)	0.5 (0.6)
Flatulence	1.5 (0.7)	1.3 (0.7)	1.4 (0.7)	0.9 (0.6)	1.5 (0.7)	1.2 (0.7)	1.4 (0.6)	1.0 (0.6)
Constipation	0.4 (0.6)	0.3 (0.4)	0.2 (0.4)	0.2 (0.4)	0.3 (0.6)	0.2 (0.4)	0.3 (0.5)	0.3 (0.4)
Diarrhoea	0.3 (0.5)	0.3 (0.5)	0.4 (0.5)	0.2 (0.5)	0.4 (0.5)	0.3 (0.5)	0.3 (0.5)	0.2 (0.4)
Loose stool	0.8 (0.7)	0.7 (0.7)	0.9 (0.6)	0.5 (0.6)	0.9 (0.7)	0.7 (0.7)	0.8 (0.6)	0.5 (0.6)
Hard stool	0.2 (0.4)	0.2 (0.2)	0.2 (0.4)	0.2 (0.3)	0.2 (0.4)	0.2 (0.2)	0.2 (0.4)	0.2 (0.3)
Urgency	0.8 (0.7)	0.7 (0.6)	1.1 (0.7)	0.6 (0.7)	1.1 (0.7)	0.7 (0.7)	0.9 (0.7)	0.6 (0.6)
Incomplete evacuation	0.8 (0.8)	0.7 (0.7)	0.9 (0.8)	0.5 (0.6)	0.8 (0.8)	0.7 (0.7)	0.8 (0.7)	0.6 (0.6)
Tiredness	1.3 (0.8)	1.3 (0.7)	1.2 (0.8)	1.0 (0.8)	1.4 (0.8)	1.2 (0.8)	1.2 (0.8)	1.0 (0.8)
Overall	1.4 (0.5)	1.2 (0.6)	1.4 (0.6)	1.0 (0.6)	1.4 (0.5)	1.2 (0.6)	1.4 (0.6)	1.0 (0.6)

Values are mean (SD) severity rated daily over seven days on a scale of 0 (absent), 1 (mild), 2 (moderate), 3 (severe)

9.16 Stool output at baseline and follow up

Stool output for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Sham diet (n=53)		Low FODMAP diet (n=51)		Placebo (n=51)		Probiotic (n=53)	
	Baseline	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**
Stool consistency	4.4 (1.1)	4.3 (1.1)	4.6 (0.8)	3.9 (1.0)	4.6 (0.9)	4.2 (1.0)	4.4 (0.9)	4.0 (1.1)
Stool frequency	14.0 (7.3)	12.9 (7.4)	15.7 (9.2)	14.0 (8.5)	14.4 (8.3)	13.8 (8.3)	15.3 (8.4)	13.1 (7.6)
% Stools normal consistency	60 (25)	61 (30)	58 (27)	67 (26)	57 (27)	64 (30)	61 (25)	64 (26)

Values are mean (SD). Stool consistency, the mean Bristol Stool Form Scale type over the 7-day period; Stool frequency, mean number of stools over the 7-day period; Stools normal consistency, proportion of stools of types 3-5 over the 7-day period

9.17 Stool output for intervention combinations

Stool output outcomes for the intervention combinations at follow up for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Sham diet + placebo (n=27)	Sham diet+ probiotic (n=26)	Low FODMAP diet + placebo (n=24)	Low FODMAP diet + probiotic (n=27)	p[#]
Stool consistency	4.3 (1.1)	4.1 (1.0)	4.3 (1.1)	3.7 (1.0)	0.137
Stool frequency	14.1 (7.9)	13.5 (8.8)	11.7 (6.7)	14.4 (8.3)	0.600
Stools normal consistency (%)	62 (31)	66 (29)	61 (28)	68 (24)	0.788

Values are mean (SD). No differences at baseline.[#]one-way ANOVA; Stool consistency, the mean Bristol Stool Form Scale type over the 7-day period; Stool frequency, mean number of stools over the 7-day period; Stools normal consistency, proportion of stools of types 3-5 over the 7-day period.

9.18 HRQOL scores at baseline and follow up

SF-36 and IBS-QOL score for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Sham (n=53)		Low FODMAP (n=51)		Placebo (n=51)		Probiotic (n=53)	
	Baseline	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**
SF-36								
Physical functioning	87.6 (18.3)	87.3 (22.3)	83.6 (22.1)	86.3 (21.3)	86.5 (20.3)	88.9 (19.2)	84.9 (20.3)	84.7 (23.8)
Role limitations due to physical health	57.1 (40.5)	55.2 (39.6)	61.3 (38.8)	70.6 (39.2)	54.9 (42.4)	62.8 (39.8)	63.2 (36.6)	62.7 (40.6)
Role limitations due to emotional problems	52.8 (39.5)	65.4 (37.5)	56.9 (42.8)	64.0 (43.1)	60.1 (38.9)	71.2 (38.3)	49.7 (42.7)	58.5 (41.3)
Energy/fatigue	38.5 (21.1)	42.6 (19.9)	41.1 (20.3)	52.1 (23.3)	37.2 (19.8)	43.9 (19.7)	42.3 (21.3)	50.4 (23.8)
Emotional wellbeing	60.5 (18.8)	63.3 (17.3)	61.8 (18.0)	68.7 (17.8)	61.5 (17.2)	66.0 (17.8)	60.8 (19.5)	65.8 (17.7)
Social functioning	67.0 (25.4)	77.8 (20.7)	64.7 (26.5)	73.3 (27.3)	65.2 (26.6)	76.0 (25.0)	66.5 (25.3)	75.2 (23.6)
Pain	56.9 (21.8)	65.0 (20.4)	50.1 (22.6)	63.5 (27.0)	50.8 (20.8)	61.0 (23.7)	56.2 (23.6)	67.5 (23.6)
General Health	53.6 (20.4)	56.2 (19.7)	51.3 (22.1)	57.5 (22.4)	52.8 (21.1)	55.8 (20.7)	52.1 (21.4)	57.8 (21.4)
IBS-QOL								
Overall	61.6 (16.8)	70.6 (18.1)	57.7 (20.1)	72.4 (19.7)	56.2 (18.5)	68.6 (20.7)	63.1 (18.1)	74.3 (16.6)
Dysphoria	58.1 (23.7)	72.2 (20.5)	54.5 (25.9)	71.9 (24.7)	52.4 (25.3)	69.6 (24.7)	60.2 (23.9)	74.4 (20.3)
Interference with activity	61.5 (20.1)	71.2 (20.6)	57.3 (24.3)	72.9 (24.2)	56.4 (21.7)	68.9 (23.3)	62.4 (22.6)	75.0 (21.1)
Body Image	58.3 (23.1)	64.1 (22.7)	55.5 (25.1)	73.2 (22.7)	51.6 (24.2)	64.8 (24.2)	62.0 (22.9)	72.2 (21.5)
Healthy worry	60.5 (22.9)	71.1 (20.8)	59.5 (22.4)	73.0 (20.0)	58.5 (23.1)	69.6 (23.3)	61.5 (22.1)	74.4 (17.0)
Food avoidance	52.5 (27.7)	57.9 (29.2)	44.4 (29.3)	51.1 (26.7)	46.1 (27.8)	53.6 (28.8)	50.9 (29.5)	55.5 (27.5)
Social reaction	63.8 (17.8)	71.7 (22.2)	61.4 (25.6)	77.5 (22.4)	58.8 (21.5)	71.2 (23.3)	66.3 (21.9)	77.7 (21.1)
Sexual	71.9 (28.9)	76.2 (28.6)	69.9 (29.1)	79.7 (24.7)	68.1 (27.1)	73.3 (29.3)	73.6 (30.5)	82.3 (23.4)
Relationships	76.3 (22.1)	80.5 (19.9)	68.5 (22.8)	81.2 (18.8)	68.8 (23.5)	77.5 (20.1)	75.9 (21.5)	84.1 (18.1)

Values are mean (SD)

9.19 HRQOL scores for intervention combinations

SF-36 and IBS-QOL scores for the intervention combinations at follow up for the intention to treat population (n=104) of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

	Sham diet + placebo (n=27)	Sham diet + probiotic (n=26)	Low FODMAP diet + placebo (n=24)	Low FODMAP diet + probiotic (n=27)	p[#]
SF-36					
Physical functioning	90.2 (18.8)	84.2 (25.4)	87.5 (20.0)	85.2 (22.6)	0.759
Role limitations due to physical health	52.8 (40.0)	57.7 (39.9)	74.0 (37.2)	67.6 (41.5)	0.226
Role limitations due to emotional problems	70.4 (36.2)	60.3 (38.9)	72.2 (41.3)	56.8 (44.2)	0.438
Energy/fatigue	38.9 (16.9)	46.4 (22.2)	49.6 (21.4)	54.3 (25.1)	0.054
Emotional wellbeing	62.7 (17.7)	63.9 (17.1)	69.8 (17.5)	67.7 (18.4)	0.441
Social functioning	78.7 (23.2)	76.9 (18.3)	72.9 (27.0)	73.6 (28.0)	0.805
Pain	62.2 (22.3)	68.0 (18.2)	59.6 (25.6)	67.0 (28.2)	0.551
General Health	52.8 (21.3)	59.8 (17.5)	59.2 (19.9)	55.9 (24.7)	0.604
IBS-QOL					
Overall	65.6 (20.3)	75.8 (14.2)	72.0 (21.0)	72.8 (18.8)	0.240
Dysphoria	67.2 (23.1)	77.4 (16.3)	72.3 (26.6)	71.5 (23.4)	0.320
Interference with activity	65.5 (22.5)	77.1 (17.0)	72.8 (24.2)	73.0 (24.6)	0.296
Body Image	59.5 (23.9)	69.0 (20.8)	70.8 (23.6)	75.2 (22.2)	0.081
Healthy worry	67.0 (24.0)	75.3 (16.4)	72.6 (22.6)	73.5 (17.8)	0.481
Food avoidance	53.4 (30.4)	62.5 (27.7)	53.8 (27.6)	48.8 (26.1)	0.352
Social reaction	67.4 (24.7)	76.2 (18.6)	75.5 (21.4)	79.2 (23.4)	0.249
Sexual	70.4 (31.8)	82.2 (24.0)	76.6 (26.4)	82.4 (23.3)	0.305
Relationships	74.4 (22.0)	86.9 (15.5)	80.9 (17.5)	81.5 (20.3)	0.134

Values are mean (SD). No differences at baseline. [#]one-way ANOVA or Welch test if homogeneity of variance was not met

	Sham diet + placebo (n=27)	Sham diet + Probiotic (n=26)	Low FODMAP diet + placebo (n=24)	Low FODMAP diet + probiotic (n=27)	p[#]
Achieving MCID n (%)	7 (26)	7 (27)	13 (54)	13 (48)	0.078

[#] Chi-squared test

9.20 Acceptability outcomes

Diet acceptability outcomes for patients with IBS (n=95) completing a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

Question	Sham diet n=48	Low FODMAP diet n=47	p[#]
Meal preparation was more difficult	58%	91%	**<0.001**
Time spent shopping for food was longer	46%	85%	**<0.001**
Time spent preparing and cooking meals and snacks was longer	23%	72%	**<0.001**
Finding suitable food choices when eating out was more difficult	79%	96%	**0.027**
The flavour of the meals and snacks was less appealing	17%	78%	**<0.001**
Grocery shopping and eating out compared to usual diet was more expensive	8%	85%	**<0.001**
Difficulty with understanding written information	0%	2%	0.489
Diet was less convenient	71%	89%	**0.039**
Diet was more troublesome/difficult	71%	91%	**0.017**
Made many changes to diet	58%	94%	**<0.001**
Missed a food or drink a lot	68%	85%	0.087
Benefits of being involved in the study outweighed the burdens of being on this diet	60%	73%	0.217

Values are proportion of patients reporting yes/neutral responses; [#]Chi-squared test

Foods most missed

Low FODMAP (% of patients)	Sham (% of patients)
Bread 34%	Rice 46%
Garlic 32%	Oats 19%
Specific vegetables 30%	Specific fruit 17%
Onion 28%	Specific vegetables 17%

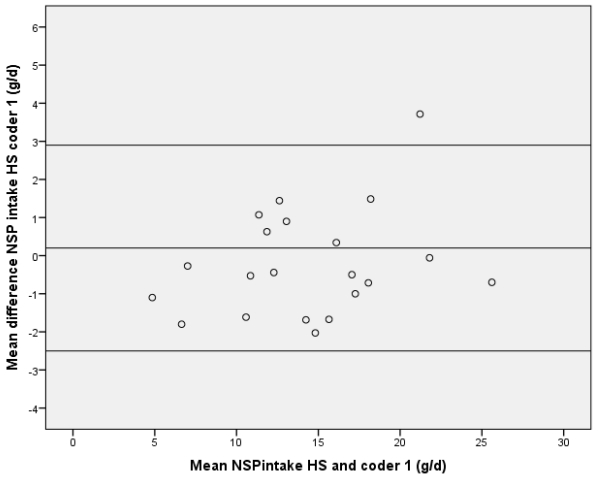
Product acceptability outcomes for patients with IBS (n=95) completing a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation

Question	Placebo n=45	Probiotic n=50	p[#]
Taking the sachets daily for 4 weeks was easy	89%	77%	0.169
I experienced a side effect from probiotic/placebo sachet	17%	19%	1.000
I believe I understand definition of probiotic	67%	64%	0.828
I would classify a probiotic as			
Standard treatment	24%	17%	
Complementary/alternative medicine	48%	60%	0.527
Neither	24%	26%	
I would take a probiotic in the future	92%	76%	0.076

[#]Chi-squared test

9.21 Bland Altman plots for inter rater agreement analysis

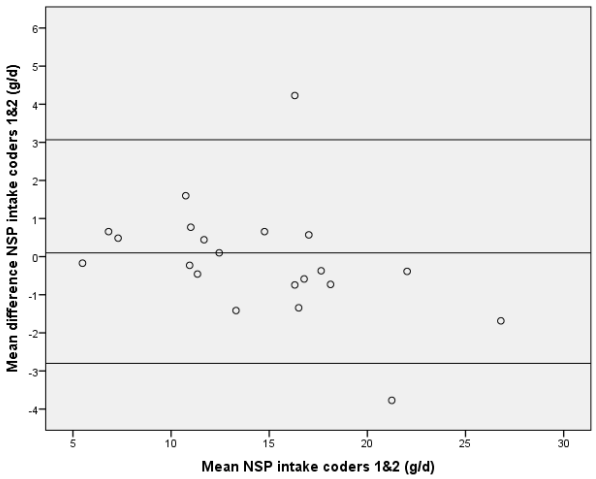
Bland Altman plots for NSP intake from 21 diet records (7 baseline, 7 sham diet, 7 low FODMAP diet) to assess inter-agreement between three coders. Diet records were from patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice



Mean +1.96 SD

Mean

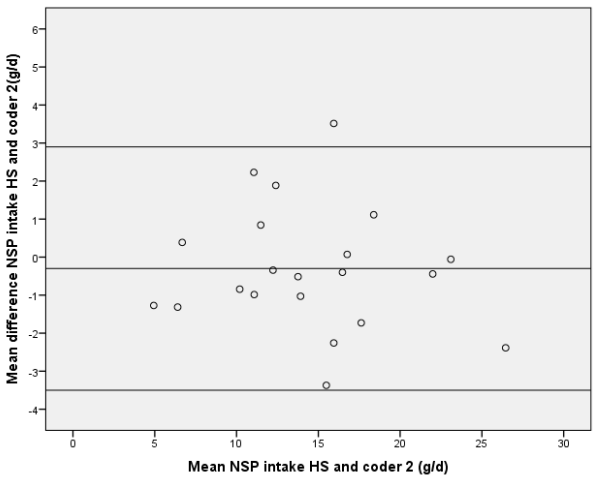
Mean -1.96 SD



Mean +1.96 SD

Mean

Mean -1.96 SD



Mean +1.96 SD

Mean

Mean -1.96 SD

9.22 Nutrient intakes from the RCT compared with NDNS and DRVs

Energy, macronutrient and micronutrient intake for males at baseline (habitual diet) (n=34) and for males in the low FODMAP group at follow up (n=16) compared with UK population intakes and dietary reference values. Patients participated in a 2x2 factorial design RCT of the low FODMAP diet and probiotic supplementation in IBS

Nutrient		Baseline	Follow up	NDNS[#]	DRV
Energy	EAR (kcal/d)	2349 (547)	2207 (440)	2124 (584)	2581-2772
Carbohydrate	Population average (% total energy intake)	43 (10)	41 (8)	45 (7)	47
Protein	RNI (g/d) (19-49yr)	93 (29)	95 (26)	85 (34)	55.5
	RNI (g/d) (50+yr)	102 (18)	102 (23)		53.3
Fat	Population average (% total energy intake)	37 (10)	37 (10)	35 (6)	33
NSP	Individual minimum (g/d)	14 (5)	13 (4)	15 (5)	12
Potassium	RNI (mg/d)	3410 (933)	3291 (905)	2975 (932)	3500
Calcium	RNI (mg/d)	909 (289)	848 (411)	875 (343)	700
Magnesium	RNI (mg/d)	338 (110)	302 (93)	283 (95)	300
Phosphorous	RNI (mg/d)	1533 (360)	1444 (401)	-	550
Iron	RNI (mg/d)	12.4 (3.1)	11.9 (2.1)	12.8 (8.2)	8.7
Zinc	RNI (mg/d)	10.6 (2.4)	9.8 (2.2)	9.7 (3.4)	9.5

DRV, Dietary reference value; NDNS, National Diet and Nutrition Survey (combined 2008/9 and 2011/12);EAR, estimated average requirement, RNI, reference nutrient intake

[#] Energy, macronutrient intakes taken from 25-49 yr cohort, micronutrient intakes from 19-65 yr cohort, all data from food sources only

Energy, macronutrient and micronutrient intake for males at baseline (habitual diet) (n=34) and for males in the low FODMAP group at follow up (n=16) compared with UK population intakes and dietary reference values. Patients participated in a 2x2 factorial design RCT of the low FODMAP diet and probiotic supplementation in IBS

Nutrient		Baseline	Follow up	NDNS[#]	DRV
Thiamin	RNI (mg/d) (19-49yr)	1.6 (0.5)	1.6 (0.5)	1.6 (0.6)	1.0
	RNI (mg/d) (50+yr)	1.7 (0.5)	1.8 (0.8)		0.9
Riboflavin	RNI (mg/d)	1.9 (0.7)	1.8 (0.5)	1.7 (0.7)	1.3
Niacin	RNI (mg/d) (19-49yr)	26 (8)	29 (10)	42 (17)	17
	RNI (mg/d) (50+yr)	30 (11)	28 (5)		16
Vitamin B6	RNI (mg/d)	2.3 (0.7)	2.5 (0.7)	2.5 (1.2)	1.4
Vitamin B12	RNI (µg/d)	6.4 (3.5)	7.4 (3.1)	5.7 (3.9)	1.5
Folate	RNI (µg/d)	264 (96)	273 (95)	287 (117)	200
Pantothenic acid	Safe intake (mg/d)	5.9 (1.8)	5.6 (2.2)	-	3-7
Biotin safe intake	Safe intake (µg/d)	43 (19)	41 (21)	-	10-200
Vitamin C	RNI (mg/d)	99 (59)	96 (57)	84 (67)	40

DRV, Dietary reference value; NDNS, National Diet and Nutrition Survey (combined 2008/9 and 2011/12);EAR, estimated average requirement, RNI, reference nutrient intake

[#] Energy, macronutrient intakes taken from 25-49 yr cohort, micronutrient intakes from 19-65 yr cohort, all data from food sources only

Energy, macronutrient and micronutrient intake for females at baseline (habitual diet) (n=70) and for females in the low FODMAP group at follow up (n=35) compared with UK population intakes and dietary reference values. Patients participated in a 2x2 factorial design RCT of the low FODMAP diet and probiotic supplementation in IBS.

Nutrient		Baseline	Follow up	NDNS[#]	DRV
Energy	EAR (kcal/d)	1831 (401)	1728 (438)	1584(463)	2079-2175
Carbohydrate	Population average (% total energy intake)	45 (12)*	43 (7)	46 (8)	47
Protein	RNI (g/d) (19-49yr)	71 (18)	72 (16)	66 (19)	45.0
	RNI (g/d) (50+yr)	66 (15)	62 (7)		46.5
Fat	Population average (% total energy intake)	38 (9)	37 (5)	34 (7)	33
NSP	Individual minimum (g/d)	14 (5)	13 (5)	13 (5)	12
Potassium	RNI (mg/d)	2759 (819)	2876 (631)	2457 (696)	3500
Calcium	RNI (mg/d)	790 (320)	739 (261)	715 (254)	700
Magnesium	RNI (mg/d)	262 (87)	250 (64)	226 (68)	270
Phosphorous	RNI (mg/d)	1152 (318)	1089 (244)	-	550
Iron	RNI (mg/d) (19-49yr)	11.0 (3.4)	10.7 (3.0)	9.4 (2.9)	14.8
	RNI (mg/d) (50+yr)	12.0 (4.1)	9.0 (1.3)		8.7
Zinc	RNI (mg/d)	7.8 (2.4)	7.5 (2.2)	7.6 (2.4)	7.0

DRV, Dietary reference value; NDNS, National Diet and Nutrition Survey (combined 2008/9 and 2011/12); EAR, estimated average requirement
RNI, reference nutrient intake
[#] Energy, macronutrient intakes taken from 25-49 yr cohort, micronutrient intakes from 19-65 yr cohort, all data from food sources only

Energy, macronutrient and micronutrient intake for females at baseline (habitual diet) (n=70) and for females in the low FODMAP group at follow up (n=35) compared with UK population intakes and dietary reference values. Patients participated in a 2x2 factorial design RCT of the low FODMAP diet and probiotic supplementation in IBS

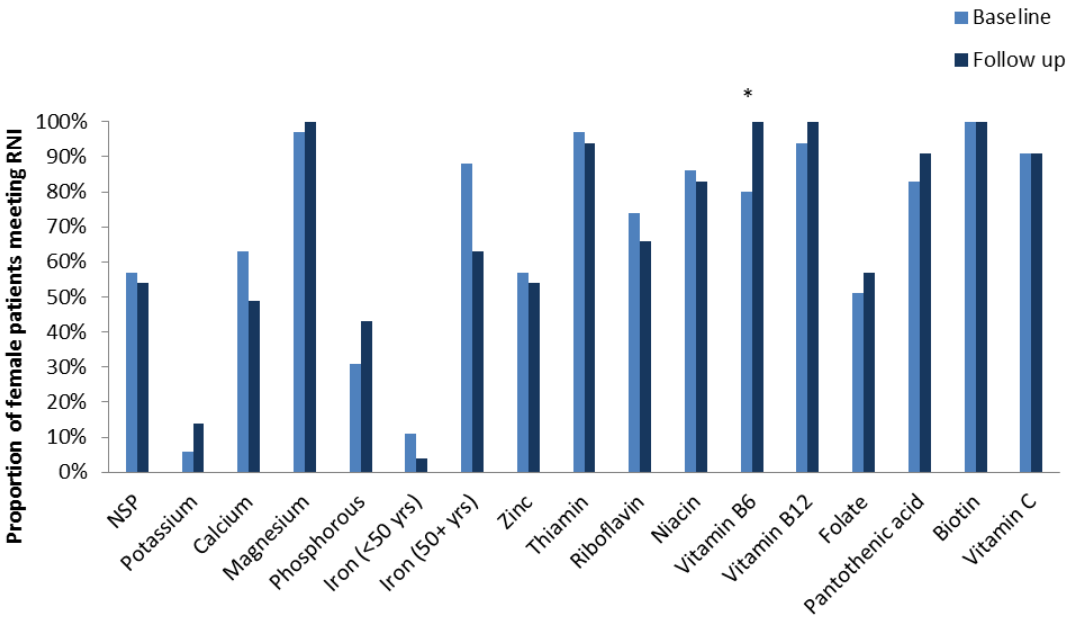
Nutrient		Baseline	Follow up	NDNS[#]	DRV
Thiamin	RNI (mg/d)	1.4 (0.5)	1.2 (0.3)	1.3 (0.4)	0.8
Riboflavin	RNI (mg/d)	1.4 (0.6)	1.3 (0.4)	1.4 (0.5)	1.1
Niacin	RNI (mg/d) (19-49yr)	18 (6)	19 (6)	32 (11)	13
	RNI (mg/d) (50+yr)	18 (5)	17 (3)		12
Vitamin B6	RNI (mg/d)	1.8 (0.7)	1.8 (0.5)	1.9 (0.8)	1.2
Vitamin B12	RNI (µg/d)	4.7 (2.8)	5.6 (2.2)	4.6 (3.2)	1.5
Folate	RNI (µg/d)	213 (84)	218 (74)	228 (84)	200
Pantothenic acid	Safe intake (mg/d)	4.3 (1.5)	4.4 (1.6)	-	3-7
Biotin	Safe intake (µg/d)	31 (15)	33 (15)	-	10-200
Vitamin C	RNI (mg/d)	92 (49)	115 (58)	82 (60)	40

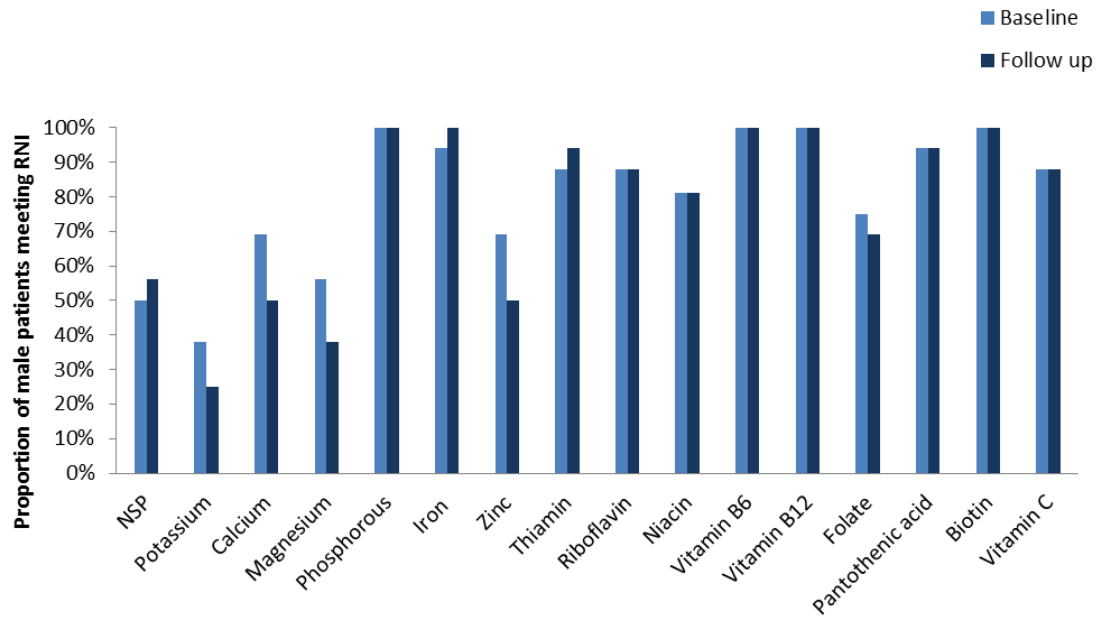
DRV, Dietary reference value; NDNS, National Diet and Nutrition Survey (combined 2008/9 and 2011/12);EAR, estimated average requirement, RNI, reference nutrient intake

[#] Energy, macronutrient intakes taken from 25-49 yr cohort, micronutrient intakes from 19-65 yr cohort, all data from food sources only

9.23 Proportion of patients in the low FODMAP diet group meeting gender-specific DRVs

Proportion of patients in the low FODMAP diet group meeting RNIs at baseline and follow up grouped by gender (females n=35, males n=16) Patients participated in a 2x2 factorial design RCT of the low FODMAP diet and probiotic supplementation in IBS
(*p<0.05, McNemar's test)





9.24 Absolute and relative abundance of microbiota for the per protocol population

Absolute abundance of microbiota (log_{10} cells/g faeces) at follow up for the per protocol population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=87)

	Sham diet (n=53)	Low FODMAP diet (n=51)	Mean difference (95% CI)	p	Placebo (n=51)	Probiotic (n=53)	Mean difference (95% CI)	p
Universal	10.9 (0.4)	10.9 (0.3)	-0.08 (-0.21, 0.06)	0.273	10.9 0.3)	10.9 (0.4)	-0.05 (-0.18, 0.07)	0.421
Bacteroides spp.	10.0 (0.7)	10.1 (0.8)	0.17 (-0.03, 0.37)	0.130	10.2 (0.5)	10.0 (0.9)	-0.19 (-0.36, -0.01)	0.072
Prevotella spp.	7.9 (1.9)	7.1 (1.8)	-0.19 (-0.50, 0.11)	0.235	7.5 (1.9)	7.5 (1.9)	-0.04 (-0.33, 0.23)	0.773
Bifidobacteria	9.2 (0.9)	8.8 (0.6)	-0.39 (-0.64, -0.13)	**0.008**	8.8 (1.0)	9.1 (0.6)	0.34 (0.05, 0.61)	**0.019**
B. longum	8.6 (0.8)	7.9 (0.8)	-0.71 (-0.96, -0.47)	**0.001**	8.3 (0.9)	8.2 (0.8)	-0.19 (-0.46, 0.07)	0.184
B. adolescentis	8.1 (1.1)	7.6 (1.1)	-0.38 (-0.82, 0.11)	0.125	7.8 (1.1)	7.9 (1.1)	-0.13 (-0.61, 0.33)	0.589
Clostridium Cluster XIVa	10.2 (0.8)	10.2 (0.6)	0.02 (-0.23, 0.27)	0.895	10.2 (0.7)	10.2 (0.8)	-0.01 (-0.26, 0.26)	0.985
Roseburia spp. & *E. rectale*	9.8 (0.5)	9.4 (0.9)	-0.22 (-0.42, -0.02)	**0.036**	9.6 (0.9)	9.6 (0.6)	0.01 (0.20, 0.21)	0.995
F.prausnitzii	9.7 (0.6)	9.6 (0.7)	-0.10 (-0.37, 0.17)	0.474	9.6 (0.8)	9.7 (0.6)	0.08 (-0.22, 0.38)	0.615
R. Bromii	8.7 (0.7)	8.6 (0.8)	-0.06 (-0.31, 0.19)	0.666	8.7 (0.7)	8.6 (0.8)	-0.13 (-0.38, 0.12)	0.293
A. muciniphila	8.0 (1.1)	7.9 (1.1)	-0.49 (-1.00, -0.01)	0.067	8.0 (1.0)	7.9 (1.2)	-0.22 (-0.70, 0.31)	0.367
Lactobacilli	7.6 (0.9)	7.7 (0.8)	-0.06 (-0.61, 0.51)	0.836	7.5 (0.9)	7.8 (0.8)	0.28 (-0.33, 0.90)	0.379

Values are mean (SD), estimated mean difference and 95% confidence interval

Relative abundance of microbiota (% of total) at follow up for the per protocol population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=87)

	Diet				Product			
	Sham diet (n=53)	**Low FODMAP diet (n=51)**	**Mean difference (95% CI)**	**P**	**Placebo (n=51)**	**Probiotic (n=53)**	**Mean difference (95% CI)**	**P**
Bacteroides spp.	18.8 (14.7)	27.5 (17.5)	8.07 (3.18, 12.99)	**0.003**	23.4 (15.7)	22.8 (17.7)	-3.63 (-8.59, 1.14)	0.141
Prevotella spp.	8.3 (16.4)	4.7 (12.2)	-2.40 (-9.16, 4.80)	0.513	5.9 (12.8)	7.0 (15.8)	5.08 (-0.73, 11.13)	0.133
Bifidobacteria	3.9 (4.9)	1.6 (1.7)	-2.27 (-3.35, -1.13)	**0.007**	2.8 (5.0)	2.8 (2.6)	0.22 (-1.16, 1.56)	0.755
B. longum	1.4 (2.1)	0.4 (1.0)	-1.06 (-1.78, -0.44)	**0.018**	1.2 (2.2)	0.7 (1.2)	-0.29 (-1.00, 0.31)	0.390
B. adolescentis	0.7 (1.0)	0.4 (0.7)	-0.49 (-0.87, -0.15)	**0.039**	0.4 (0.6)	0.7 (1.1)	-0.01 (-0.30, 0.29)	0.958
Clostridium Cluster XIVa	23.4 (15.1)	25.9 (13.6)	1.70 (-4.68, 7.50)	0.558	23.3 (14.0)	25.8 (14.8)	3.36 (-2.37, 8.92)	0.238
Roseburia spp. & *E. rectale*	8.3 (7.0)	7.8 (8.9)	-0.42 (-3.34, 2.69)	0.783	7.3 (6.4)	8.7 (9.1)	1.22 (-1.67, 3.88)	0.409
F. prausnitzii	8.8 (9.1)	9.2 (10.0)	0.29 (-4.13, 4.51)	0.907	8.4 (9.4)	9.5 (9.6)	1.17 (-3.13, 5.41)	0.606
R. Bromii	0.9 (0.8)	1.1 (1.1)	0.33 (-0.04, 0.674)	0.080	1.1 (1.2)	1.0 (0.8)	-0.29 (-0.64, 0.12)	0.165
A. muciniphila	0.4 (0.5)	0.4 (0.9)	0.09 (-0.34, 0.66)	0.780	0.5 (0.9)	0.3 (0.4)	-0.49 (-1.13, 0.05)	0.305
Lactobacilli	0.2 (0.6)	0.2 (0.2)	-0.06 (-0.44, 0.19)	0.719	0.2 (0.7)	0.2 (0.2)	-0.10 (-0.55, 0.18)	0.633

Values are mean (SD), estimated mean difference and 95% confidence interval

9.25 Absolute and relative abundance of microbiota for intervention combinations

Absolute abundance of microbiota (log_{10} cells/g faeces) at follow up for the combination interventions of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet + placebo (n=27)	Sham diet + probiotic (n=26)	Low FODMAP diet + placebo (n=24)	Low FODMAP diet + probiotic (n=27)	p[#]	Between groups comparisons p
Universal	10.9 (0.4)	10.9 (0.4)	10.9 (0.3)	10.9 (0.4)	0.886	-
Bacteroides spp.	10.1 (0.5)	10.0 (0.7)	10.3 (0.4)	10.1 (0.9)	0.303	-
Prevotella spp.	7.9 (1.9)	7.8 (1.9)	7.1 (1.7)	7.1 (1.8)	0.285	-
Bifidobacteria	8.8 (1.3)	9.2 (0.8)	8.6 (0.7)	8.9 (0.6)	**0.037***	SH+Pro vs LFD+Pla* **0.020**
B. longum	8.6 (1.0)	8.4 (0.9)	8.0 (0.9)	8.0 (0.8)	0.077	-
B. adolescentis	8.2 (1.0)	8.1 (1.1)	7.3 (0.9)	8.9 (1.1)	0.059	-
Clostridium Cluster XIVa	10.1 (0.7)	10.1 (0.9)	10.0 (0.7)	10.3 (0.6)	0.731	-
Roseburia spp. & *E. rectale*	9.8 (0.5)	9.8 (0.5)	9.3 (1.0)	9.5 (.06)	**0.049**	>0.05
F. prausnitzii	9.7 (0.6)	9.7 (0.7)	9.4 (1.1)	9.7 (0.5)	0.453	-
R. Bromii	8.7 (0.7)	8.7 (0.7)	8.5 (1.0)	8.6 (0.8)	0.639	-
A. muciniphila	7.9 (1.1)	7.9 (1.1)	8.1 (0.9)	7.7 (1.1)	0.906	-
Lactobacilli	7.4 (0.9)	7.6 (0.9)	7.6 (1.1)	7.6 (0.8)	0.894	-

Values are mean (SD) [#]ANOVA with Tukey post-hoc tests and Welch tests with Games-Howell post hoc where homogeneity of variances was violated.
No differences at baseline.
*Welch test with Games-Howell post-hoc test. SH, sham diet; LFD, low FODMAP diet; Pro, probiotic; Pla, placebo.

Relative abundance of microbiota (% of total) at follow up for the combination interventions of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet + placebo (n=27)	Sham diet + probiotic (n=26)	Low FODMAP diet + placebo (n=24)	Low FODMAP diet + probiotic (n=27)	p	Between groups comparisons p
Bacteroides spp.	18.9 (11.8)	20.1 (17.0)	33.5 (15.8)	26.0 (17.7)	**0.006**	SH+Pla vs LFD+Pla 0.008 SH+Pro vs LFD+Pla 0.019
Prevotella spp.	8.0 (14.6)	8.5 (17.5)	3.3 (8.5)	6.0 (14.4)	0.613	-
Bifidobacteria	3.8 (5.8)	3.8 (3.2)	1.3 (1.8)	1.7 (1.5)	**0.005***	Sh+Pro vs LFD+Pla 0.008* Sh+Pro vs LFD+Pro 0.022*
B. longum	1.5 (2.4)	1.1 (1.4)	0.7 (1.2)	0.3 (0.3)	**0.009***	>0.05*
B. adolescentis	0.6 (0.6)	0.8 (1.3)	0.1 (0.2)	0.5 (0.8)	**0.002***	SH+Pla vs LFD+Pla 0.012*
Clostridium Cluster XIVa	22.7 (18.2)	24.6 (16.9)	22.1 (14.6)	27.6 (12.4)	0.591	-
Roseburia spp. & *E. rectale*	7.7 (5.1)	10.2 (9.6)	6.9 (7.3)	7.8 (9.4)	0.502	-
F. prausnitzii	8.0 (5.4)	10.7 (10.8)	10.3 (12.1)	8.6 (7.3)	0.682	-
R. Bromii	0.9 (1.0)	1.0 (0.8)	1.3 (1.8)	1.0 (0.9)	0.543	-
A. muciniphila	0.4 (0.5)	0.3 (0.4)	0.6 (1.4)	0.2 (0.4)	0.762*	-
Lactobacilli	0.3 (0.9)	0.1 (0.2)	0.2 (0.3)	0.2 (0.2)	0.882	-

Values are mean (SD) [#]ANOVA with Tukey post-hoc tests and Welch tests with Games-Howell post hoc where homogeneity of variances was violated.
No differences at baseline
*Welch test with Games-Howell post-hoc test. SH, sham diet; LFD, low FODMAP diet; Pro, probiotic; Pla, placebo.

9.26 Stool SCFA at baseline and follow up

Stool SCFA concentration (µmol/g faeces) and stool pH for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet (n=53)		Low FODMAP diet (n=51)		Placebo (n=51)		Probiotic (n=53)	
	Baseline	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**
Total SCFA	119.9 (48.8)	124.0 (69.4)	115.1 (45.1)	98.6 (43.7)	122.6 (52.3)	108.6 (42.8)	112.7 (40.9)	113.1 (71.9)
Acetate	70.8 (30.4)	73.1 (37.1)	69.1 (30.8)	58.2 (25.9)	73.6 (35.0)	64.8 (27.4)	66.5 (25.1)	66.8 (43.6)
Butyrate	21.4 (10.4)	21.5 (15.8)	18.2 (8.6)	15.7 (9.5)	20.5 (10.6)	17.9 (10.1)	19.2 (8.7)	19.4 (15.9)
Propionate	20.9 (11.6)	20.9 (16.2)	20.5 (9.5)	18.0 (9.7)	21.5 (12. 6)	18.9 (8.3)	20.0 (8.3)	20.1 (17.0)
Valerate	2.5 (2.4)	2.4 (1.5)	2.4 (1.2)	2.0 (1.3)	2.5 (1.3)	2.2 (1.2)	2.5 (1.3)	2.2 (1.6)
Isobutyrate	2.3 (1.3)	2.7 (1.8)	2.7 (1.1)	2.6 (1.4)	2.5 (1.2)	2.7 (1.7)	2.5 (1.3)	2.6 (1.6)
Isovalerate	2.0 (1.0)	2.2 (1.4)	2.2 (0.9)	2.0 (1.1)	2.0 (0.9)	2.2 (1.2)	2.1 (1.0)	2.1 (1.4)
pH	6.7 (0.5)	6.7 (0.5)	6.7 (0.4)	6.8 (0.5)	6.6 (0.5)	6.8 (0.5)	6.7 (0.5)	6.8 (0.5)

Values are mean (SD)

9.27 Stool SCFA for intervention combinations

Stool SCFA concentration (µmol/g faeces) and stool pH for the intervention combinations at follow up for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet + placebo (n=27)	Sham diet + probiotic (n=26)	Low FODMAP diet + placebo (n=24)	Low FODMAP diet + probiotic (n=27)	p[#]
Total SCFA	122.7 (42.8)	122.9 (89.9)	92.8 (37.6)	103.7 (48.7)	0.115
Acetate	74.7 (28.2)	71.4 (45.1)	53.7 (22.0)	62.3 (28.8)	0.052
Butyrate	20.6 (10.4)	22.4 (20.1)	14.8 (9.1)	16.5 (10.0)	0.075
Propionate	19.9 (8.0)	21.8 (21.8)	17.7 (8.7)	18.3 (10.7)	0.692
Valerate	2.3 (1.1)	2.5 (1.9)	2.0 (1.4)	1.9 (1.2)	0.337
Isobutyrate	2.3 (1.4)	2.1 (1.5)	2.0 (1.1)	2.1 (1.2)	0.906
Isovalerate	2.8 (1.8)	2.6 (1.7)	2.6 (1.5)	2.5 (1.4)	0.968
pH	6.7 (0.6)	6.8 (0.5)	6.9 (0.5)	6.8 (0.6)	0.524

Values are mean (SD) [#] Kruskal-Wallis Test for SCFA and one-way ANOVA for pH. No differences at baseline

9.28 Proportion of patients with microbiota below the detection limit

Proportion of patients (%) with IBS in the intention to treat population participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation with microbiota below the detection limit

	Sham diet (n=53)		Low FODMAP diet (n=51)		p[#]	p*	Placebo (n=51)		Probiotic (n=53)		p[#]	p*
	Baseline	Follow up	Baseline	Follow up			Baseline	Follow up	Baseline	Follow up		
Universal	0	0	0	0	-	-	0	0	0	0	-	-
Bacteroides spp.	2	2	2	0	1.000	1.000	2	2	2	0	1.000	0.490
Prevotella spp.	28	11	29	8	1.000	0.742	29	12	28	8	1.000	0.522
Bifidobacteria	6	4	0	2	0.243	1.000	4	6	2	0	0.614	0.114
B. longum	2	9	0	12	1.000	0.758	2	8	0	7	0.490	0.527
B. adolescentis	38	34	41	37	0.841	0.838	41	31	38	40	0.841	0.418
Clostridium Cluster XIVa	0	0	0	0	-	-	0	0	0	0	-	-
Roseburia spp. & *E. rectale*	2	2	0	0	1.000	1.000	2	2	0	0	0.490	0.490
F. prausnitzii	0	0	0	0	-	-	0	0	0	0	-	-
R. Bromii	2	2	0	0	1.000	1.000	2	2	0	0	0.490	0.490
A. muciniphila	49	38	57	59	0.440	0.049	57	51	49	47	0.440	0.695
Lactobacilli	43	30	53	31	0.433	1.000	44	53	51	9	0.563	<0.001

[#] Comparison of baseline proportions (Chi-squared) *Comparison of follow up proportions (Chi-squared)

9.29 Absolute and relative abundance of microbiota at baseline and follow up

Absolute abundance of microbiota (log_{10} cells/g faeces) at baseline and follow up for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet (n=53)		Low FODMAP diet (n=51)		Placebo (n=51)		Probiotic (n=53)	
	Baseline	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**
Universal	10.9 (0.4)	10.9 (0.4)	10.9 (0.3)	10.9 (0.3)	10.9 (0.4)	10.9 (0.3)	10.9 (0.3)	10.9 (0.4)
Bacteroides spp.	10.2 (0.7)	10.0 (0.6)	10.2 (0.8)	10.1 (0.7)	10.2 (0.7)	10.2 (0.5)	10.2 (0.8)	10.1 (0.8)
Prevotella spp.	8.4 (1.9)	7.8 (1.9)	7.7 (2.0)	7.1 (1.7)	8.1 (2.0)	7.5 (1.8)	8.0 (1.9)	7.4 (1.9)
Bifidobacteria	8.7 (1.2)	9.0 (1.1)	8.8 (1.1)	8.8 (0.7)	8.7 (1.2)	8.7 (1.1)	8.8 (1.1)	9.1 (0.7)
B. longum	8.5 (1.1)	8.5 (0.9)	8.5 (1.2)	8.0 (0.8)	8.5 (1.1)	8.3 (1.0)	8.5 (1.2)	8.2 (0.9)
B. adolescentis	8.4 (0.9)	8.2 (1.1)	8.2 (1.1)	7.6 (1.0)	8.1 (1.0)	7.8 (1.1)	8.4 (1.1)	8.0 (1.1)
Clostridium Cluster XIVa	9.9 (0.8)	10.1 (0.8)	9.9 (0.8)	10.2 (0.6)	9.9 (0.8)	10.1 (0.7)	9.9 (9.8)	10.2 (0.7)
Roseburia spp. & *E. rectale*	9.8 (0.5)	9.8 (0.5)	9.6 (0.8)	9.4 (0.8)	9.6 (0.8)	9.5 (0.8)	9.7 (0.5)	9.6 (0.6)
F. prausnitzii	9.9 (0.5)	9.7 (0.6)	9.7 (0.9)	9.5 (0.8)	9.7 (0.7)	9.6 (0.8)	9.8 (0.7)	9.7 (0.6)
R. bromii	8.6 (0.7)	8.7 (0.7)	8.4 (1.0)	8.5 (0.9)	8.4 (0.9)	8.6 (0.8)	8.6 (0.9)	8.6 (0.8)
A. muciniphila	7.9 (1.0)	7.9 (1.1)	8.0 (1.0)	7.9 (1.0)	7.6 (1.1)	7.9 (1.0)	8.2 (0.8)	7.8 (1.1)
Lactobacilli	7.1 (0.9)	7.6 (0.9)	7.1 (0.9)	7.6 (0.9)	7.2 (0.9)	7.5 (1.0)	7.0 (0.9)	7.6 (0.9)

Values are mean (SD)

Relative abundance of microbiota (% of total) at baseline and follow up for the intention to treat population of patients with IBS participating in a 2x2 factorial design RCT of low FODMAP dietary advice and probiotic supplementation (n=104)

	Sham diet (n=53)		Low FODMAP diet (n=51)		Placebo (n=51)		Probiotic (n=53)	
	Baseline	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**	**Baseline**	**Follow up**
Bacteroides spp.	31 (24)	20 (15)	34 (22)	30 (17)	31 (21)	26 (16)	34 (24)	23 (17)
Prevotella spp.	16 (28)	8 (16)	12 (26)	5 (12)	17 (33)	6 (12)	11 (18)	7 (16)
Bifidobacteria	3 (5)	4 (5)	3 (4)	2 (2)	3 (4)	3 (5)	3 (4)	3 (3)
B. longum	2 (3)	1 (2)	2 (3)	<1 (1)	2 (4)	1 (2)	2 (3)	1 (1)
B. adolescentis	1 (1)	1 (1)	1 (2)	<1 (1)	1 (1)	<1 (1)	1 (2)	1 (1)
Clostridium Cluster XIVa	17 (16)	24 (17)	18 (15)	25(13)	19 (17)	22 (16)	16 (12)	26 (15)
Roseburia spp. & *E. rectale*	10 (8)	9 (8)	8 (7)	7 (8)	9 (7)	7 (6)	9 (7)	9 (9)
F. Prausnitzii	11 (9)	9 (9)	11 (12)	9 (10)	11(13)	9 (9)	11 (13)	10 (9)
R. bromii	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
A. muciniphila	<1 (<1)	<1 (<1)	<1 (<1)	<1 (1)	<1 (<1)	<1 (1)	<1 (<1)	<1 (<1)
Lactobacilli	<1 (<1)	<1 (1)	<1 (<1)	<1 (<1)	<1 (<1)	<1 (1)	<1 (<1)	<1 (<1)

Values are mean (SD)